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Physioecological Responses to Climate Changes in Polar Regions

May 26 - 28, 2010
Korea Polar Research Institute
Incheon, Korea

Sung Gu Lee and Sang Heon Lee
Editors

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Korea Polar Research Institute

Symposium Program

May 26, 2010		
08:30 - 09:30	**Registration**	
Opening Ceremony & Welcoming Address		*Chair: Sang Heon Lee*
09:30 - 10:10	***Welcome Address***	
10:10 – 10:20	***Group Photo***	
10:30 - 11:00	***Coffee Break***	
Plenary Lecture		
11:00 - 12:00	Koji Shimada	Hydrographic changes in the Pacific Sector of the Arctic Ocean
12:00 - 13:00	***Lunch***	
Session 1: Current status & changes in polar ecosystems		*Chair: Hyung Chul Shin*
13:00 - 13:20	Sang Heon Lee	Current status of phytoplankton community in the Western Arctic Ocean
13:20 - 13:40	Jacobo Martin	Carbon cycling in the coastal Beaufort sea: first results of the malina 2009 survey
13:40 - 14:00	Hyoung Min Joo	Latitudinal variation of phytoplankton community and carbon biomass in the western Arctic Ocean
14:00 - 14:20	Hans Uwe Dahms	The ephemeral habitat of polar sea ice – current studies and future challenges
14:20 - 14:40	Su Jong Jeong	Is it still greening in Arctic region?
14:40 - 15:00	***Coffee Break***	
Special Lecture		*Chair: Sung Gu Lee*
15:00 - 16:00	Paul Gordon Falkowski	The Once and Future Ocean
Session 2: Polar ocean & sea-ice ecosystems		*Chair: Ho Kyung Ha*
16:00 - 16:20	Meibing Jins	Modeling study of the Arctic sea ice and ocean primary production and model validation the western Arctic
16:20 – 16:40	Kokubun Nobuo	Multi-dimensional measurement of penguin foraging behavior using data loggers
16:40 - 17:00	Phil Hwang	Combining satellite remote sensing with sea ice mass balance study
17:00 - 18:00	***Coffee Break & Poster Session***	
18:00 - 20:00	***Banquet***	

May 27, 2010		
Plenary Lecture		*Chair: Soon Gyu Hong*
09:00 - 10:00	Stephen Craig Cary	A Landscape Scale Approach to Predicting Biodiversity in the Dry Valleys, Southern Victoria Land, Antarctica
10:00 - 10:20	***Coffee Break***	
Session 3: Stress responses of cold-adapted organisms		*Chair: Sung Gu Lee*
10:20 - 10:40	Suhk Neung Pyo	Immunomodulating activity of polar lichens
10:40 - 11:00	Marcelo Gonzalez	Immune response in the Antarctic sea urchin (Sterechinus neumayeri): Cellular and molecular characterization under thermal stress
11:00 - 11:20	Hyun Cheol Oh	Screening and identification of PTP1B inhibitory Antarctic secondary metabolites for the treatment of type 2 diabetes
11:20 - 11:40	Dong Gyu Jo	Anti-diabetic effects of lobaric acid isolated from Antarctic lichen.
11:40 - 12:00	Ha Na Im	Cold shock proteins of polar microorganisms
12:00 - 12:20	Kwang Hwan Jung	Color Tuning of Microbial Rhodopsins on the surface of Arctic and Antarctic Ocean
12:20 - 13:30	***Lunch***	
Session 4: Polar terrestrial & Marine ecosystems		*Chair: Joung Han Yim*
13:30 - 13:50	Larry Hinzmam	
13:50 - 14:10	Parfenova Valentina	Adaptation mechanisms of Lake Baikal microorganisms to extreme environments, their taxonomic diversity and biological potential
14:10 - 14:30	Tae Seok Ahn	Microbial Diversity in Lake Khuvsgul And Lake Uvs, Mongolia And in Freshwater Sponge of Lake Baikal, Russia
14:30 - 14:50	Hans Uwe Dahms	Studies in secondary production – copepod feeding
14:50 – 15:10	Xuehua Cui	Feeding ecology of dominant groundfish in the northern Bering Sea determined by stomach contents and stable isotope analysis
15:10 – 15:30	Terkina Irina	Microbiological analyses of extremophilous communities of Antarctica, the arctic zone and lake Baikal
15:30 - 17:40	***Coffee Break & Poster Session***	
18:00 - 20:00	***Welcome Dinner***	

May 26-27, 2010		
Poster Session		
PS-01	Byung Hoon Jung	Comparative Study of Microbial Rhodopsins between Arctic and Antarctic Ocean
PS-02	Carlo Papucci	Two main Italian investigations in the marine environment of the Svalbard region (1996-2010), in an era of climate change. Past, present and future
PS-03	Eun Hye Kim	Kopria litus gen. nov., sp. nov., of the Family Oxalobacteraceae, Isolated from Antarctic Coastal Seawater
PS-04	Eun Jung Choy	Carbon and nitrogen isotope ratios in the Antarctic limpet Nacella concinna from rocky coastal habitats, Marian Cove, King George Island
PS-05	Eun Jung Choy	Flow cytometric observation of picoplankton community structure in the Russian Chukchi Sea in 2009
PS-06	Ha Ju Pack	Characterization of cold-active β-N-acetylglucosaminidase from Arctic bacterium Pseudoalteromonas issachenkonii KOPRI 22718
PS-07	Hans Uwe Dahms	UVR effects on biota with emphasis on polar regions
PS-08	Ho Sung Yoon	Low-temperature propagation of an isogenic Arctic cyanobacterium for a genetic source of consistent biomass production
PS-09	Hyoung Seok Lee	Pyrosequence analysis of the antarctic hairgrass Deschampsia antarctica under various abiotic stresses
PS-10	Hyun Ju Noh	Diversity of Polyketide Synthase Genes in lichen Cladonia spp
PS-11	Jong Won Han	Gene expression profiling of cold stress responses using expressed sequence tag (EST) from a freshwater green alga, Spirogyra varians
PS-12	Jung Ho Hong	The diversity of Harpacticoid Copepods in the polar regions.
PS-13	Liping Jiao	Persistent Toxic Substances in Remote Lake and Coastal Sediments from Svalbard, Norway: Levels, Sources and Fluxes
PS-14	Mi Sun Yun	The effects of light and nutrient enrichment on the primary productivity in the Canada Basin
PS-15	Min Gui Jung	Protein adaptation in polar environment: a comparative study of α-tubulin seqences in mesophilic and psychrophilic polar microalgae
PS-16	Min Jung Kim	Molecular characterization of cold responsive protein AnF48_RPL11 from Antarctic micro green alga AnF48
PS-17	Min Seok Kwak	Apoptotic and anti-inflammatory effect of methanolic extract from freshwater green alga, Spirogyra varians in chondrocytes and cancer cell
PS-18	Pil-Sung Kang	Plastid transformation of an arctic moss, Aulacomnium turgidum
PS-19	Sang Hee Kim	Single nucleotide polymorphisms based phylum specific PCR amplification technique (SPAT): its application to analyze environmental microbial eukaryotic community
PS-20	Sang Heon Lee	Phytoplankton productivity in the Russian Chukchi Sea in 2009
PS-21	Se Jong Han	Comparative production of prodigiosin by fed-batch culture of Hahella chejuensis M3349 using various carbon sources
PS-22	Sun Yong Ha	UV- absorbing compounds (Mycosporine like amino acids) distribution and uptake rate in DaSan station, Arctic regions

PS-23	Sun Young Kim	Redescription of Laackmanniella naviculaefera (Laackmann, 1907) Kofoid&Campbell, 1929 (Ciliophora: Tintinnida) from Antarctic water
PS-24	Sung Gu Lee	In vitro antioxidant activity of Ramalin from Antarctic lichen Ramalina terebrata in murine macrophage RAW 264.7 cells
PS-25	Yong Won Kim	Carbon Dynamics of Forest Floor and Stem in Black Spruce Forest Soils, Interior Alaska
PS-26	Yung Mi Lee	Cultured bacterial diversity and human impact on alpine glacier cryoconite
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PLENARY LECTURE Ⅰ

HYDROGRAPHIC CHANGES IN THE PACIFIC SECTOR OF THE ARCTIC OCEAN

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1. INTRODUCTION

After the minimum record of summer sea ice extent in 2007, the oceanographic structure has changes into different state from that in pre-IPY. We briefly review the hydrographic changes from the surface mixed layer to the Fram Strait Branch of the Atlantic Water.

2. CHANGES IN SURFACE MIXED LAYER AND PACIFIC SUMMER WATER

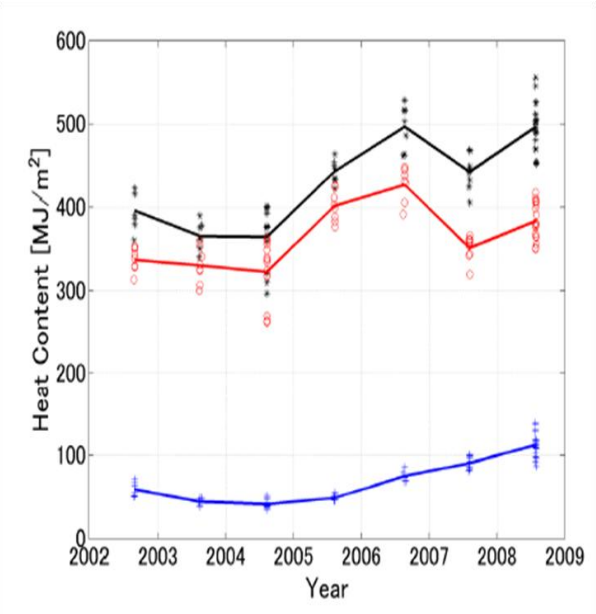

Figure 1. Ocean heat content(74-76°N, 150-158°W).

Black: 5-150m, Blue: 5-30m, Red: 30-150m.

Before IPY, changes of the heat content above the main halocline (< 150m) were mainly controlled by the heat transportation of Pacific Summer Water driven by sea ice motion (Figure 1). Surface warming (<30m) owing to solar radiation was not so large relative to the internal ocean warming (30-150m). After 2007, the surface warming has initiated associated with earlier disappearances of sea ice cover. Figure 2 shows the vertical section of temperature long 150oW in the Canada Basin. Before IPY, single temperature maximum associated with Pacific Summer Water appeared in upper layer above the main halocline. After 2007, another temperature maximum water was identified near the surface. This maximum was formed by surface warming by solar radiation. Another important feature associated with surface warming is an appearance of temperature minimum layer centered around 50m deep between the two temperature maximums. This temperature minimum was not formed by lateral advection of winter shelf waters, but was formed by vertical convection yielding upward heat flux from the Pacific Summer Water layer to the

surface mixed layer. In fact, the temperature of Pacific Summer Water decreased. Simultaneously, thinning of the Pacific Summer Water layer was also observed.
This implied that the upward ocean heat flux after 2007 was considerably increased and wintertime sea ice formation was considerably decreased. As the result, sea ice thickness at the melt onset became not to be enough thick to survive although summer season.

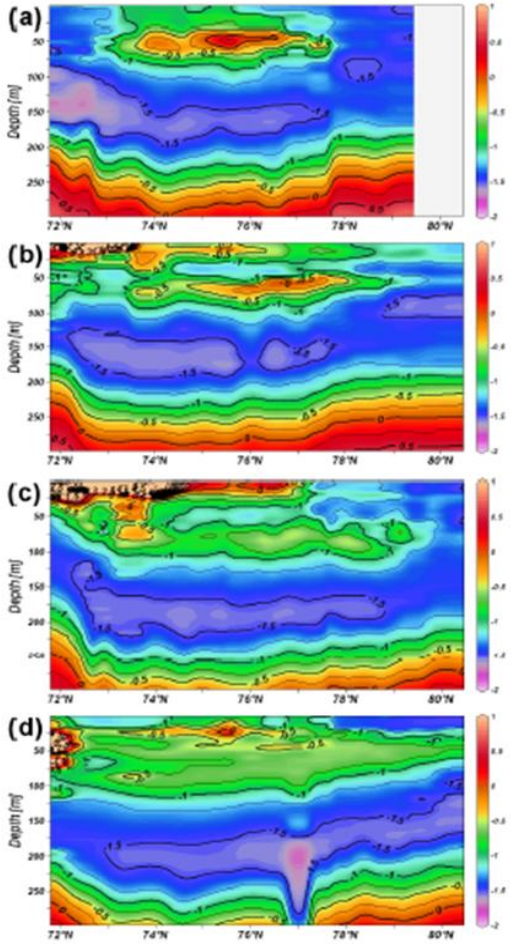

Figure 2. Vertical section of potential temperature along 150°W. (a) 2006, (b) 2007, (c) 2008, (d) 2009

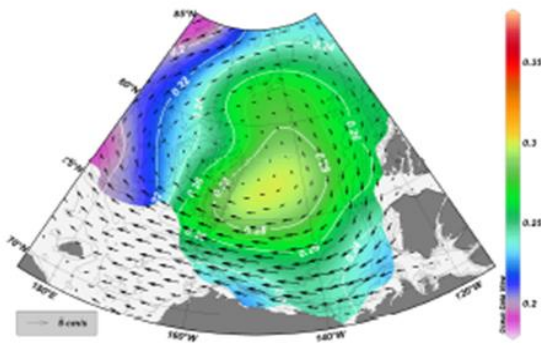

Figure 3. Dynamic height at 100dbar referred to 800 dbar and sea ice motion (climatological mean for November to May).

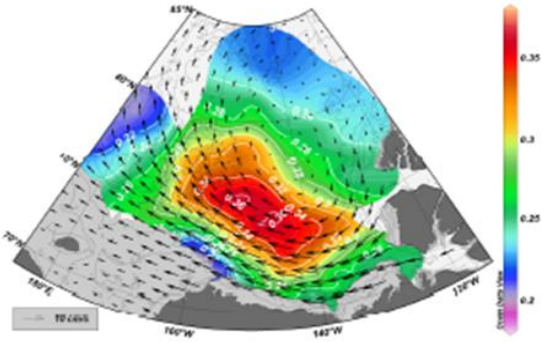

Figure 4. Dynamic height at 100dbar referred to 800 dbar (2008) and sea ice motion (November 2007 to May 2008).

3. DISTORTION OF OCEAN CIRCULATION PATTERN AROUND THE CHUKCHI

Borderland area

The circulation pattern of oceanic Beaufort Gyre is not identical to that of sea ice motion in the Pacific sector of the Arctic Ocean. Figure 3 shows sea ice motion overlying the ocean dynamic height at 150 dbar relative to 800dbar showing the geostrophic ocean circulation of Pacific Water. The sea ice motion over the Chukchi borderland is westward, but the underlying ocean current is northward. In this region the direction of ocean current and ice motion completely different. This means that simple dynamics of Ekman convergence can not explain the spatial pattern of oceanic Beaufort Gyre, especially on real shelf-basin interaction between the Chukchi Sea and the Canada Basin. The northward ocean current is associated with the large amplitude of seafloor elevation. In the Arctic Ocean, restoring force by planetary beta effect was

small, then the annual cycle of baroclinic Rossby Wave does not have wave solution. Excited Barotropic Rossby Wave with annual frequency associated with seasonal variation of Beaufort High can propagate westward. In the presence of finite amplitude seafloor elevation, the wave energy of the barotropic Rossby wave is transferred to that of baroclinic mode. Since the baroclinic mode cannot propagate as Rossby wave, the energy remained around the large amplitude of sea floor topography. This means the ocean current around the large topography is established by energy transform from the barotropic wave.

4. CHANGES IN SPREADING PATHWAY OF PACIFIC WINTER WATER INTO THE CANADA BASIN

As mentioned above, the Chukchi Borderland plays a role of western boundary just like as in the mid latitude ocean. Recently the discrepancy of the spatial pattern of the circulation between ice motion and ocean current became large. Figure 4 shows the ice motion and ocean dynamic height in 2008. The northward current in 2008 was considerably amplified, some significant changes in deliveries of Pacific Water was expected. In fact, considerable volume of Pacific Winter Water was delivered northward into the Canada Basin along the Chukchi Borderland (Figure 5). Before IPY period, pathway of Pacific Winter Water was eastward along the northern Chukchi slope and entered the deep Canada Basin near the southern end of the Northwind Ridge (Figure 5) . The difference between in pre-IPY and 2008 indicate that the wind (sea ice motion) driven circulation jointly with sea floor topography in upper ocean overcame the buoyancy driven circulation along the seafloor topography. This change is not only important for the ocean physics but rather important nutrient distribution in the Pacific sector of the Arctic Ocean. Now the Chukchi Borderland area is key area to understand the catastrophic changes between the Arctic climate and ecosystem.

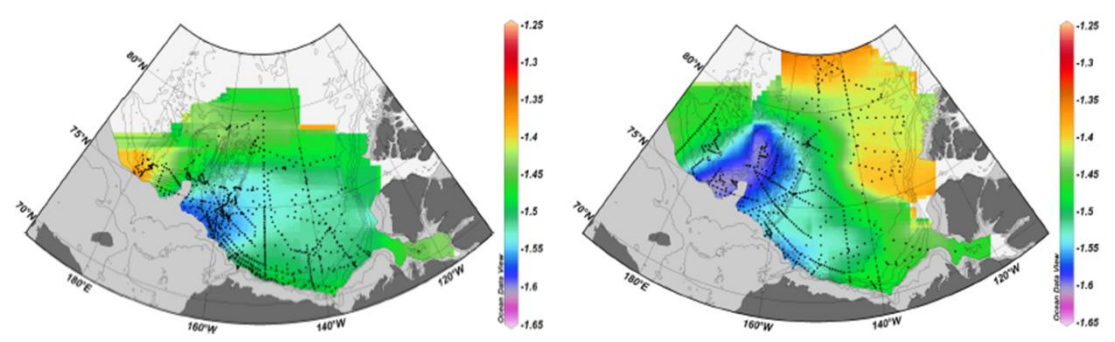

Figure 5. Potential temperature on S=33.1. Left panel: 2002-2004, Right panel: 2008.

5. CHANGES IN ATLANTIC WATER

The warm temperature anomaly of Fram Strait Branch of the Atlantic Water that entered the Arctic Ocean around 1990 was completely spread into the entire Canada Basin (Figure 6). Now the difference of water mass properties between central basin and rim of the basin (steep slope) becomes small. Double diffusive interleaving tends to be weak.

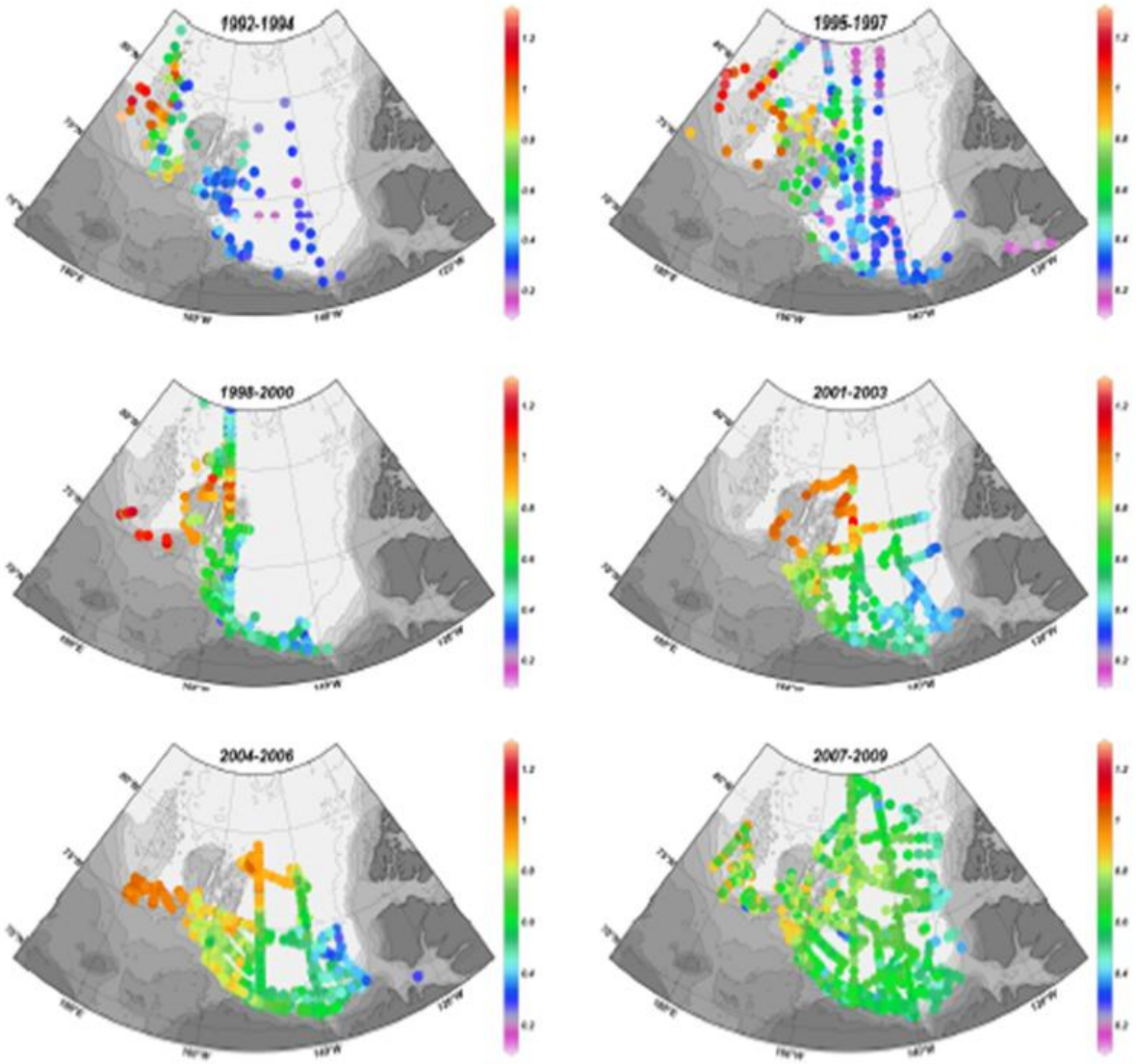

Figure 6. Potential temperature on 27.9 σ_θ near the temperature maximum of Fram Strait Branch of the Atlantic Water since 1992.

SESSION Ⅰ

CURRENT STATUS
AND
CHANGES IN POLAR ECOSYSTEMS

CURRENT STATUS OF PHYTOPLANKTON COMMUNITY IN THE WESTERN ARCTIC OCEAN

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INTRODUCTION

Over the past several decades, higher temperatures have decreased the extent of sea-ice cover as well as its thickness in the Arctic Ocean. This way the overall amount of perennial sea ice in the Arctic Ocean, especially in the western Arctic Ocean became reduced (Perovich et al., 2009). The removal of seasonal and permanent sea ice cover altered several important processes, such as the depth of mixing, stratification, light penetration, nutrient supply, temperature-related processes, and possibly photochemical reactions (Tremblay et al., 2008; Codispoti et al., 2009; Lee et al., 2010). These recent changes in climate and ice conditions could change the patterns and the total amount of carbon production of phytoplankton and consequently the production at higher trophic levels. However, it is controversial whether climate change conditions enhance or reduce the overall production in the Arctic Ocean. Since the responses of phytoplankton production to current environmental conditions could be different in different regions, three main regions of the western Arctic Ocean – the northern Bering Sea, Chukchi Sea, and the deep Canada Basin - were separated by their geological locations (Fig. 1).

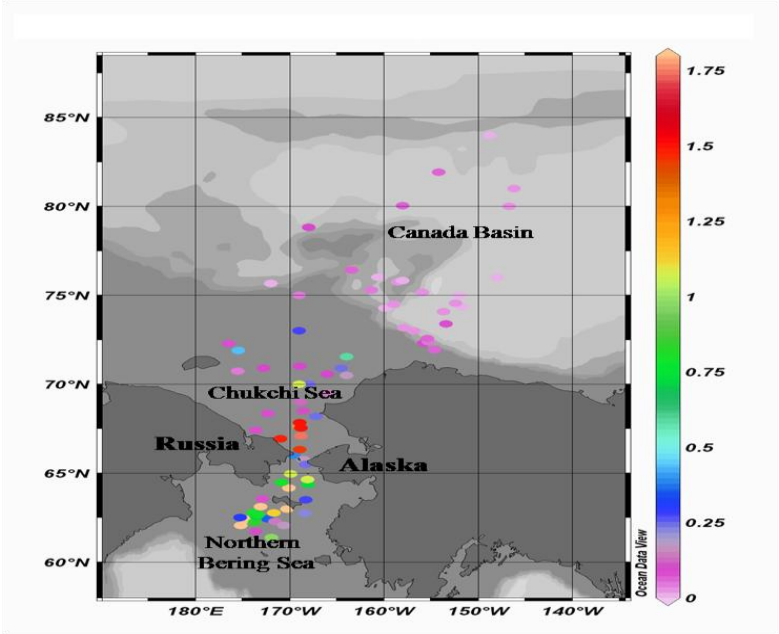

Fig 1. Recent daily carbon uptake rates (g C m^{-2}) integrated from surface to 1 % light depth in the Western Arctic Ocean. Data from Lee and Whitledge (2005), Lee et al (2007), Lee et al (2010),

RESULTS AND DISCUSSION

-Northern Bering Sea

The northern Bering Sea is a seasonally ice-covered shelf which is strongly influenced by the advection of cold, nutrient-rich Pacific water from the edge of the deep Bering Sea basin (Springer 1988; Grebmeier et al. 2006a). Recently measured daily carbon uptake rates range from 0.1 to 3.9 g C m^{-2} d^{-1} (mean ± S.D. = 1.1 ± 1.2 g C m^{-2} d^{-1}) and from 0.2 to 2.0 g C m^{-2} (mean ± S.D. = 0.9 ± 0.6 g C m^{-2} d^{-1}) for the southern part and northern part, respectively, in the northern Bering Sea. These data were recorded during the Healy cruise in 2007 (Fig. 1; Lee et al. submitted), based on a 15-h photoperiod (Hansell and Goering 1990) and hourly uptake rates. For comparative purposes with results from previous studies, the annual carbon production was roughly estimated with the assumption of a 120-day growing season in this region (Hansell et al. 1993; Sambrotto et al. 1984). The estimated annual carbon production rate of phytoplankton based on the average daily production rate was only 120 g C m^{-2} (based on an average daily production rate of about 1.0 g C m^{-2} d^{-1} considering the whole cruise period from the Healy cruise in 2007. These estimated annual rates are 2 to 3 folds lower than those in previous studies in the northern Bering Sea more than a decade ago (250-470 g C m^{-2} - see Hansell and Goering 1990; Springer and McRoy 1993; Springer et al. 1996). Recently, Grebmeier et al. (2006b) found that geographic displacement of marine mammal populations coincided with a reduction of benthic prey populations in the region throughout a period of 1988-2004. They hypothesized that ecosystem change and declining productivity are reducing food supply to benthic prey, thus affecting apex predators (Grebmeier et al. 2006b). The recent lower phytoplankton production and subsequent decline of benthic biomass might partly be due to a decrease in the phytoplankton biomass transported from lower latitudes, because the amount of phytoplankton production largely depended on the carbon biomass (Lee et al. submitted).

-Chukchi Sea

Based on 100 growing days (Springer and McRoy 1993), the recent annual production of phytoplankton is between 10 to 150 g C m^{-2} with a mean of 73 g C m^{-2} for the southern Chukchi Sea, while the mean annual production for the whole Chukchi Sea including the northwestern part is somewhat lower (55 g C m^{-2}) (Lee et al. 2007). In comparison, the estimated averages of annual primary production rates for the whole Chukchi Sea are 148 and 170 g C m^{-2} from Zeeman (1992) and Korsak (1992) respectively. Although the average values of their production rates are comparable to the highest values in the study of Lee et al. (2007), the recent average annual production is 2 or 3 times lower than the previous estimates. The average production based on an interval of 120 days is estimated as 144 g C m^{-2} y^{-1} by Lee et al. (2007) which is still lower than that estimated by Hansell et al. (1993) (576-720 g C m^{-2} y^{-1}) or Sambrotto et al. (1984) (324 g C m^{-2} y^{-1}). The recent lower rates might be the result of seasonal, annual, and/or geographical variations in primary productivity in the Chukchi Sea. Those variations are well known in this area and are mostly attributed to different water masses and thus nutrient concentrations (Springer and McRoy 1993; Springer 1988; Hansell and Goering 1990). The lower productivity observed might be an indication of the recent decline in primary production of lower latitude regions in the Bering Sea. Northward flow in the northern Bering Sea originated from the North Pacific Ocean (Bering Sea) flushes into the Chukchi Sea through the Bering Strait (Danielson et al. 2006). Therefore, a decline of phytoplankton biomass from lower latitudes such as the Bering Sea could cause a decrease in phytoplankton production in higher latitudes such as the northern Bering and Chukchi Seas.

-Deep Canada Basin

In general, the total phytoplankton biomass represented by chlorophyll-a at surface is very low (<0.2 mg Chl a m^{-3}), whereas the relatively higher concentrations (<1 mg Chl a m^{-3}) of the chlorophyll-maximum layer is prominent between 40 to 60 m water depths in the Canada Basin depending on season (Lee and Whitledge 2005; Lee et al. 2010). The maximum photosynthetic rates are also found at the chlorophyll-maximum layer depths in this basin (Lee and Whitledge 2005). Their daily primary production rates in the open water ranged from 79 to 145 mg C m^{-2}, with a mean of 106 mg C m^{-2} in the deep Canada Basin from mid-August to early September 2002. These rates are much lower than those estimated in the Eastern Canadian Arctic from 227-450 mg C m^{-2} (Grainger 1975; Harrison et al. 1982), but are comparable to those from a recent study in the Canada Basin (Cota et al. 1996). In contrast, the rates below sea ice with snow cover are much lower those in open waters. The daily production rates below the sea ice (of about 2.3 m thickness) ranged from 2.6 to 26.8 mg C m^{-2}, with a mean of 11.3 mg C m^{-2} in a study by Lee and Whitledge (2005). The authors explained this with the very low light intensity beneath the ice. In comparison, the rates under thinner sea ice (with a thickness of about 1.5 m) ranged from 20.4 to 178.3 mg C m^{-2} d^{-1} in the Canada Basin from 27 June to 26 July 2005, with a mean of 59.5 mg C m^{-2} d^{-1} (Lee et al. 2010). This was significantly higher than the mean uptake rate found under thicker sea ice in 2002 (Lee and Whitledge 2005), even though seasonal variations of phytoplankton production rates in the Arctic Ocean are considered (English 1961; Pautzke 1979). In fact, Gosselin et al. (1997) found that the mean daily carbon uptake rate of phytoplankton was 35 mg C m^{-2} d^{-1} under a sea ice thickness of about 2.0-m in the Canada Basin from 26 July to 26 August 1994. Since their uptake rates were estimated under a sea ice cover of 90-100%, their rate could be rather higher than that under solid sea ice cover of 100%.

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CARBON CYCLING IN THE COASTAL BEAUFORT SEA: FIRST RESULTS OF THE MALINA 2009 SURVEY

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Climate change is expected to deeply affect the Arctic pelagic ecosystems via ice cover reduction and increase of freshwater discharge. Important but mostly unknown consequences are to follow for the downward export of particulate matter and the carbon cycling in general. This situation has caused a growing need of field observations to monitor the complex and rapidly changing Arctic environment and to enlarge the dataset available to modelers.

In the framework of the project MALINA (www.obs-vlfr.fr/Malina), a multidisciplinary study lead by France and Canada, an intensive survey of the co ntinental shelf off the Mackenzie River delta in the Beaufort Sea was done durin g August 2009 (Figure 1). One of the main contributions of IAEA-MEL to this international effort is the study of the vertical flux of particulate material and of organic carbon.

A drifting line equipped with four PPS3 Technicap sediment traps and current-meters (immerged at 40, 85, 145 and 200 m nominal depths) was deployed at selected sites of the Canadian Beaufort Sea between August 14 and August 25 2009. Mooring deployments lasted for 28-50 hours and targeted the shelf-break and the slope along the Beaufort-McKenzie continental margin, as well as the edge between the Mackenzie Shelf and the Amundsen Gulf.

Also, the water column was sampled using large volume Challenger *in-situ* pumps and Niskin bottles mounted on a CTD-Rosette. These samples were used to assess the disequilibrium between the natural radionuclide ^{234}Th and its parent radionuclide ^{238}U.

First results obtained in the field during the 2009 MALINA survey are presented, including direct measurements of mass and organic carbon downward flux by means of

drifting traps, and estimates of particulate organic carbon export through the ^{234}Th: ^{238}U disequilibria. Also, initial results on lipid biomarkers on the particles collected by the pumps are reported.

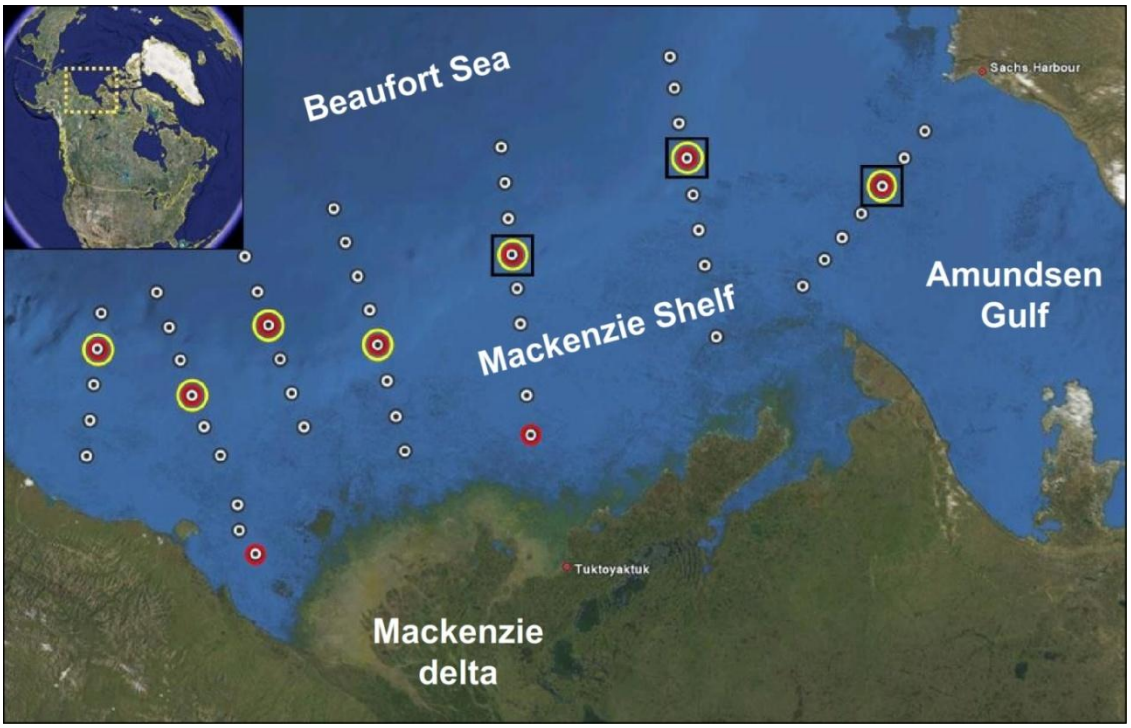

Figure 1. *Study area showing the position of sampling stations. The dense dotted grid corresponds to the general sampling scheme of the MALINA 2009 survey, including vertical casts with a multisensor CTD probe and water sampling. Sampling carried out by IAEA-MEL and presented in this contribution includes: vertical profiles of water sampling for total Th-234 and U-238 (red circles), in situ pumping to collect particles of different sizes (yellow circles) and deployments of drifting mooring lines equipped with sediment traps at 3-4 depths (black squares).*

LATITUDINAL VARIATION OF PHYTOPLANKTON COMMUNITIES AND CARBON BIOMASS IN THE WESTERN ARCTIC OCEAN

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Short Title: Phytoplankton communities along latitude in the western Arctic Ocean

ABSTRACT

A number of recent studies showed that photosynthetic eukaryotes are an active and often dominant component of Arctic algal assemblages. In order to place these observations in a large-scale context, samples were collected to investigate the community structure and biomass of phytoplankton along a transect in the western Arctic Ocean. The transect included 37 stations at surface and sub-chlorophyll-a maximum (SCM) depths in the Bering Sea, Chukchi Sea and Canadian Basin from July 19 to September 5, 2008. Phytoplankton (>2 μm) were identified and counted by light microscopy. Cluster analysis of abundances and biomass revealed different assemblages over the shelf, slope and basin regions.

The spatial distribution of phytoplankton was heterogeneous along the transect. Phytoplankton communities were composed of 71 taxa representing Dinophyceae, Cryptophyceae, Bacillariophyceae, Chrysophyceae, Dictyochophyceae, Prasinophyceae and Prymneosiophyceae. The most abundant species were nano-pico sized phytoplankton at surface and SCM depths of most stations, but the second dominant species were variable by stations. Overall, the phytoplankton community was strongly dominated by 10 general, dominant species were *Thalassiosira* sp., *Chaetoceros* sp. and unidentified nano-pico phytoplankton such as *Dinobryon belgica* and *Cryptomonas* sp. Phytoplankton abundance reached a maximum of 8.29×10^6 cells mL^{-1} at station R09(surface). Nano and pico sized phytoplankton were tentatively dominant in the Bering Sea, whereas diatoms and nano sized plankton were major taxonomy communities in the Bering Strait and Chukchi Sea. From the western Bering Sea to the Bering Strait, the abundance and biomass of phytoplankton were getting greater and species diversity was richer, but after passing through the Bering Strait these parameters decreased providing a latitudinal gradient to the central Arctic. Although nano and pico phytoplankton were the important contributors for increasing cell abundance in the study area, their contents of Chl-a and biomass were not higher than those of micro size cells. These results might be thought that micro size phytoplankton such as small prasinophytes and larger haptophytes and diatoms co-dominated in near-surface assemblages in largely ice-free waters in the western Arctic Ocean in summer.

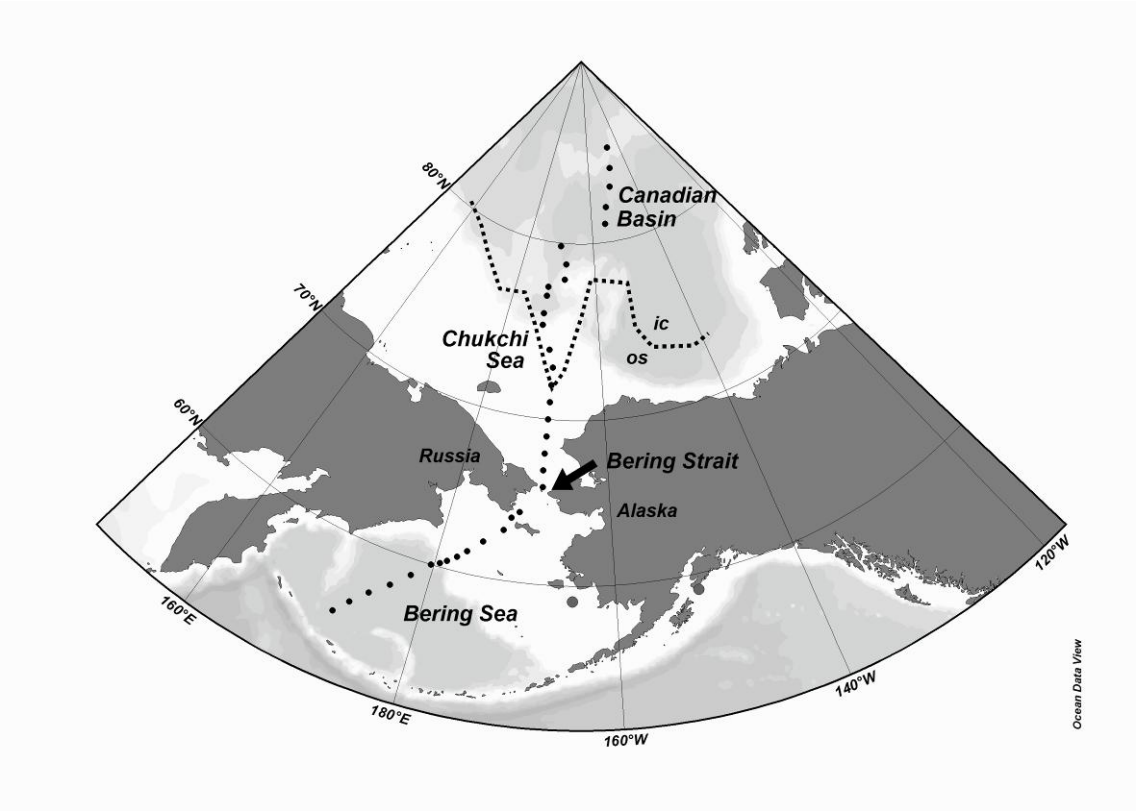

Fig. 1. *Location of the sampling stations in the Arctic sea visited from 19 July to 5 September 2008. os: open sea; ic: ice-covered sea. Four oceanographic provinces were identified: Bering Sea (stations BR23 to NB21), Bering Strait (stations BS21 to R01), Chukchi Sea (stations R03 to N01), Canadian Basin (stations D80 to B85).*

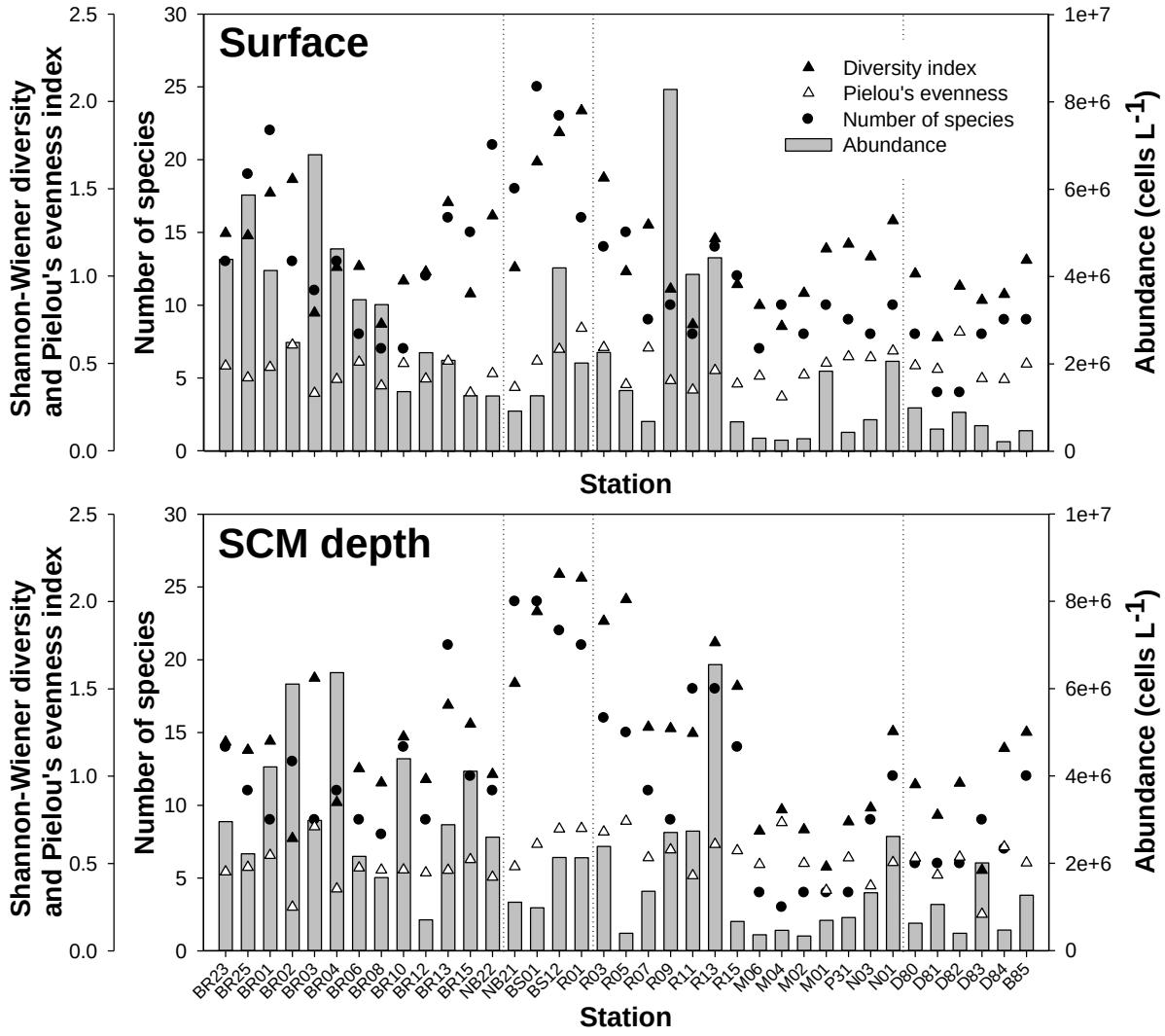

Fig. 2. *Shannon-Wiener diversity, evenness, number of species and phytoplankton abundance according to latitude (from low latitudes (left) to high latitudes (right)).*

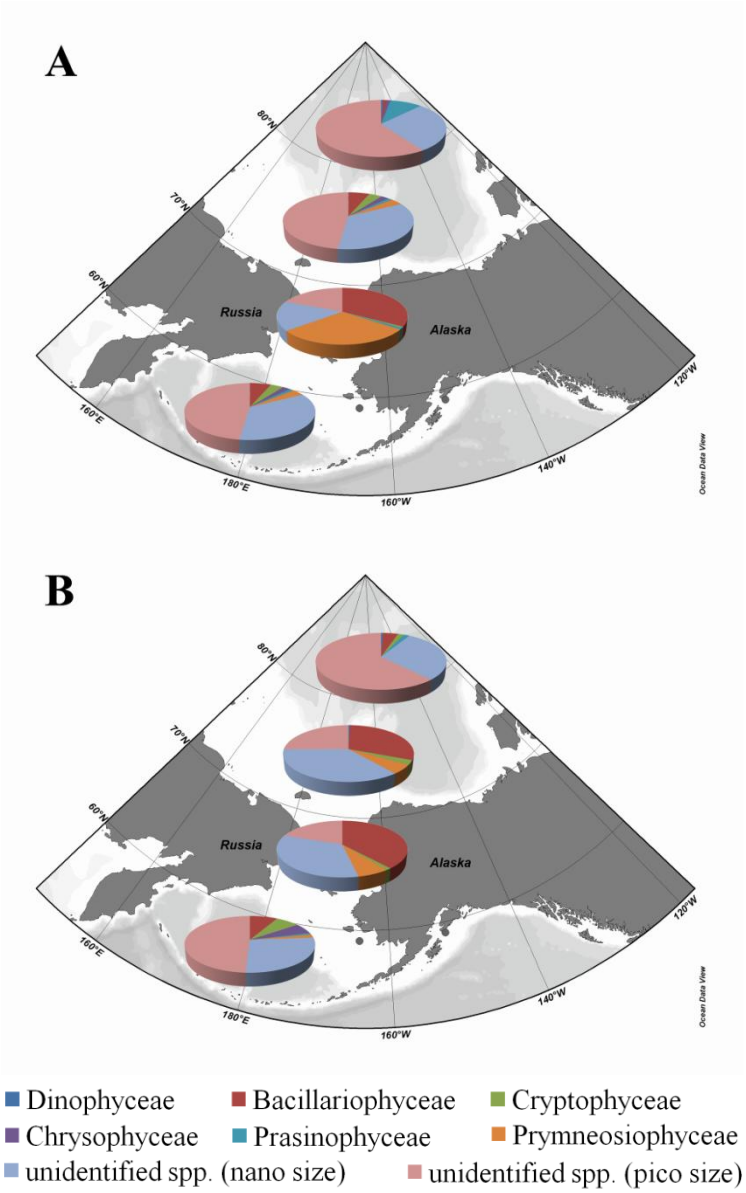

Fig. 3. *Composition of phytoplankton communities by abundance in the study area (A: surface, B: SCM depth). The pie chart provide relative abundances of major taxa: Dinophyceae, Bacillariophyceae, Cryptophyceae, Chrysophyceae, Prasinophyceae, Prymneosiophyceae, unidentified nano-sized phytoplankton, unidentified pico-sized phytoplankton.*

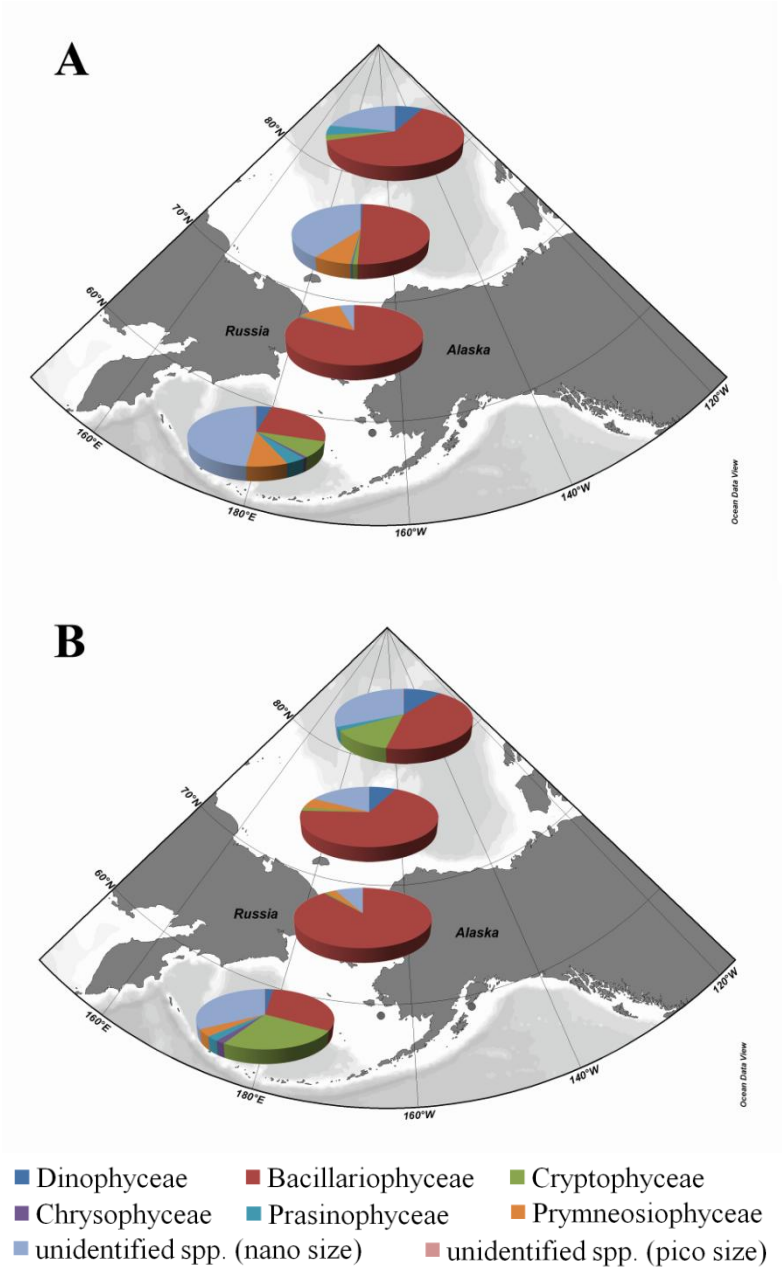

Fig. 4. *Composition of phytoplankton communities by biomass in the study area (A: surface, B: SCM depth). The pie chart provide relative abundances of major taxa: Dinophyceae, Bacillariophyceae, Cryptophyceae, Chrysophyceae, Prasinophyceae, Prymneosiophyceae, unidentified nano-sized phytoplankton, unidentified pico-sized phytoplankton.*

THE EPHEMERAL HABITAT OF POLAR SEA ICE – CURRENT STUDIES AND FUTURE CHALLENGES

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In the pack ice zone where sea ice is reformed every year after melting away in summer, the ice habitat and its biotic communities are ephemeral. Therefore, the sympagic biota have to recolonize the newly formed annual ice either from the sea bottom, the water column or from a marginal fast ice belt off the coast. Above deeper water recolonization from the benthos is unlikely. Respective biota, if not completely neustonic must be able to invade newly formed sea ice from the plankton by vertical migration. This also holds for several sympagic harpacticoid copepods. Whereas most harpacticoid representatives show a strictly benthic biology, a minor portion is exclusively planktonic and even fewer are only known as inhabitants of sea ice. Adults of the sympagic *Drescheriella racovitzai* display pronounced swimming/ floating behavior in the laboratory indicating its pelagic intermezzo during ice-free periods. Nineteen copepod species were found in Arctic sea ice, nine of which belong to the Harpacticoida. We showed the Antarctic sea ice harpacticoid *Drescheriella glacialis* to be the first polar invertebrate metazoan that has been cultured throughout its life cycle for many generations in the laboratory. We provided its demographic characteristics on the basis of a laboratory cohort study and correlative field data. When compared to its closest temperate-zone relatives belonging to the genus *Tisbe*, *D. glacialis* shows temperature compensation of developmental and reproductive rates. A genuine r-strategist in every respect, it does not fit established trends for polar invertebrates, but appears well adapted to the peculiar spatio-temporal variability of the sea ice habitat. To date, our knowledge of the life cycles of polar invertebrate metazoa is based on field censuses and the extrapolation of physiological measurements. The vast majority of polar invertebrates are characterized by long (>l year) life cycles, delayed maturity, low total reproductive investment but high investment per offspring, and highly seasonal reproduction and recruitment. Such trends are well documented, even though the relative importance of low temperature vs. resource seasonality and of adversity- vs. "K"-selection, in shaping polar life histories remains a matter of debate. In exhibiting r-selected traits as well as continuous reproduction, this sympagic harpacticoid copepod proves to be strikingly atypical of the general trend. Our findings support the idea that low temperatures *per se* do not prevent the evolution of "fast" life cycles if an unusual ecological template selects for them. Sea ice is seen here as a fascinating study area of adaptations to the extremes.

IS IT STILL GREENING IN ARCTIC REGION?

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Terrestrial vegetation of Arctic region is rendered as one of the important feedback components of global climate system. Thus, exact evaluation of the vegetation dynamics to changes in climate is inclusive procedure for understanding future climate change. Previous studies reported that increase in temperature during the last decade of 20th century lead to increase in vegetation growth (i.e., greening) particularly in high-latitude region [Keeling et al., 1996; Myneni et al., 1998]. After these observational evidences, many climate model studies focused on the climatic consequences of this greening. However, most observational findings on the arctic greening heavily relied on the data before 21th century. In this study, by using the more extended period of satellite-measured normalized difference vegetation index (NDVI) from 1982 to 2008, we investigate the changes in vegetation greenness between the period 1982-1999 and 1982-2008. Although most of features of changes in NDVI obtained from 1982 to 1999 are generally consistent with the previous studies, however, the features during 1982-2008 changed quite distinctively. It is found that NDVI in the Arctic region has significantly increased for 1982-1999, but decreased after then (Fig.1 and Fig. 2). This greening and browning features are clearly seen in North America and eastern Eurasia. Detailed results and possible environmental factors causing the browning in recent decade will be discussed in the talk.

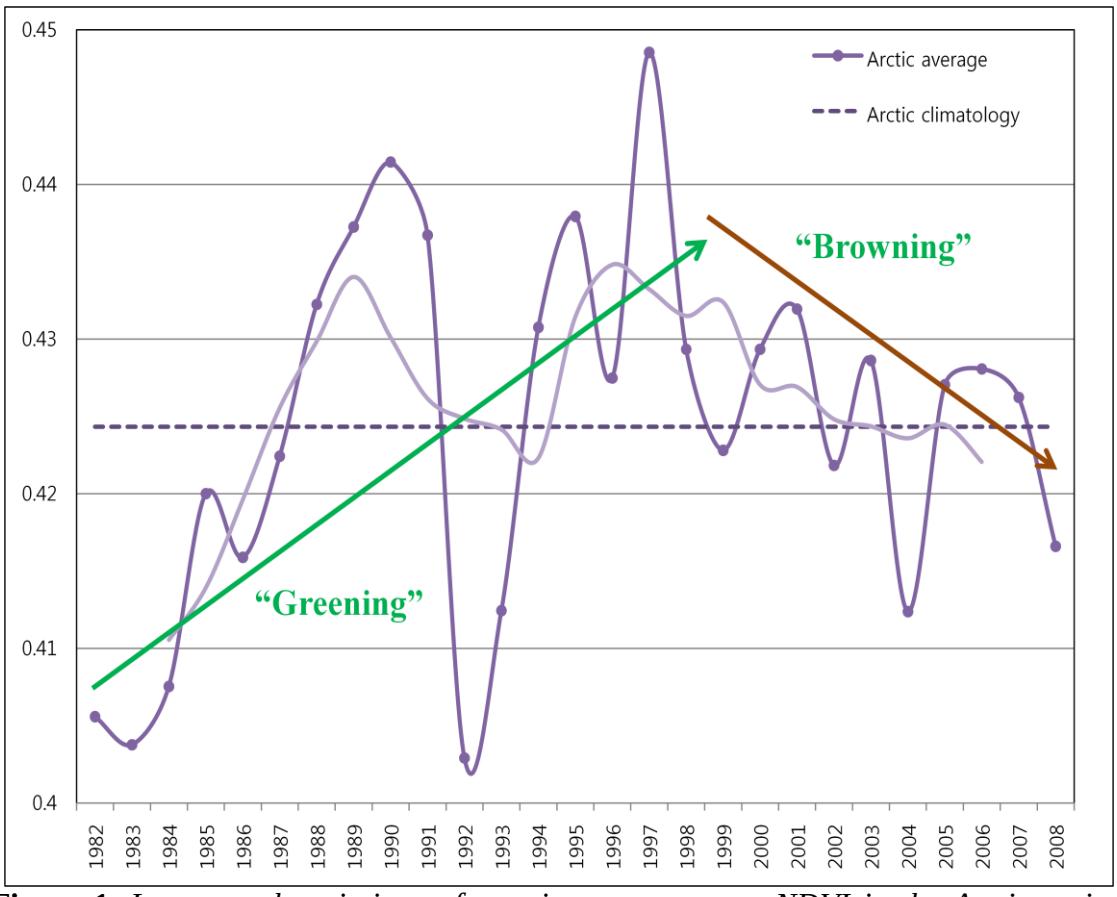

Figure 1. *Interannual variations of growing season mean NDVI in the Arctic region during the period 1982-2008.*

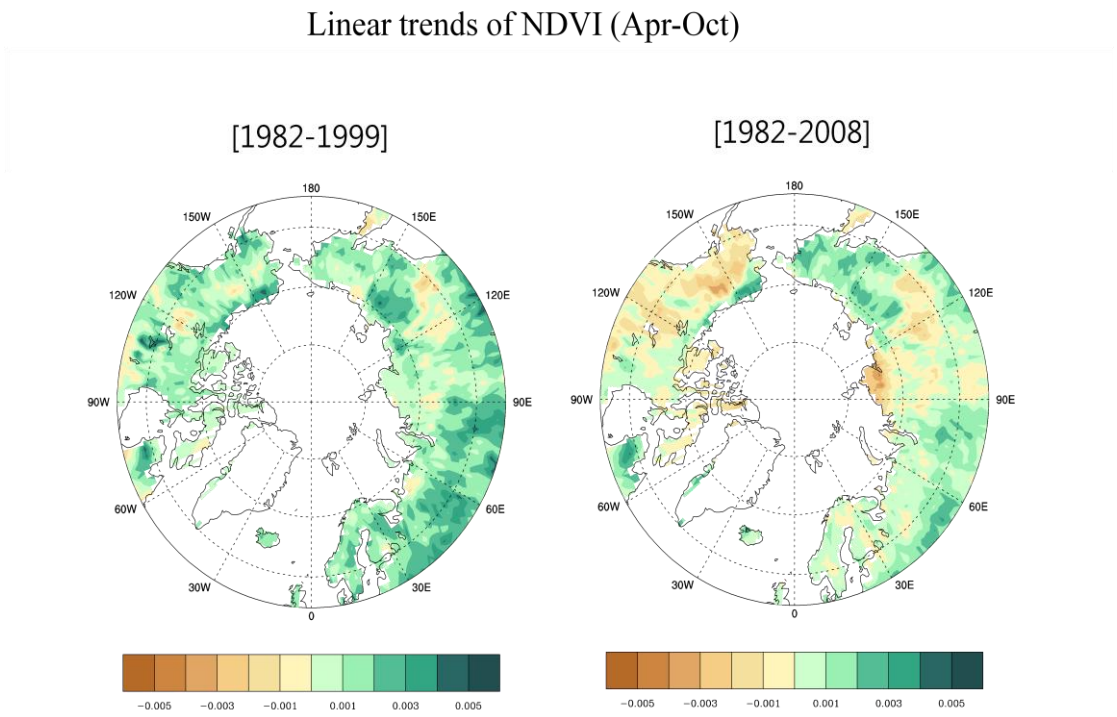

Figure 2. *Spatial distributions of the linear trends of average growing season (April-October) NDVI for 1982─1998 and for 1982─2008.*

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Myneni, R.B., C.D. Keeling, C.J. Tucker, G. Asrar, and R.R Nemani, Increased plant growth in the northern high latitudes from 1981 - 1991, Nature, 386, 698-702, 1997.

SPECIAL LECTURE

THE ONCE AND FUTURE OCEAN

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The ocean has been a feature of Earth's surface for at least 4 of the past 4.5 billion years, and has provided the primary environment for the evolution of microbes that drive the biogeochemical cycles on Earth. Over this extremely long period of time, the ocean has witnessed extreme changes, ranging from complete coverage with ice to extensive periods when there was no ice at all; periods of extraordinary extinction of animal life, to periods of extreme evolutionary radiation of animals. Throughout all of Earth's history, the ocean has served as the primary backbone of life on the planet; and the core metabolic processes have been successfully transferred across vast stretches of geological time. Humans, in contrast, evolved only about 200,000 years ago, and in that short period of time have come to successfully outcompete and plunder many of Earth's resources. Over the past 100 years, in particular, we have increasingly altered the trophic structure of the ocean as well as its physical circulation and chemical properties. While human impacts will surely alter ecosystem functions, especially in polar seas, the core metabolism of the ocean will go on. Rather, ironically, humans are the fragile species that will lose capabilities of using the ocean as a source of food and novel molecules. Our future is intimately tied to that of the ocean. We have to begin viewing the oceans as key component of the Earth system; one that we cannot live without.

SESSION Ⅱ

POLAR OCEAN
AND
SEA-ICE ECOSYSTEMS

MODELING STUDY OF THE ARCTIC SEA ICE AND OCEAN PRIMARY PRODUCTION AND MODEL VALIDATION THE WESTERN ARCTIC

Meibing Jin, Clara Deal (IARC/UAF), Sang H. Lee (KOPRI)
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ABSTRACT

In the Arctic Ocean, both phytoplankton and sea ice algae are important contributors to primary production and the arctic food web. An ice algal ecosyst em model, which has been lacking in previous arctic ecosystem models, was add ed to fully couple with the global physical model POP-CICE (Parallel Ocean Pro gram- Los Alamos Sea Ice Model) and the open-ocean pelagic ecosystem model. The physical model captured the seasonal and interannual variations of northern hemispheric sea ice extent and area measured by satellite remote sensing for the model period of 1992 to 2007. The model results showed a reasonable mean sea sonal cycle of ice algal production from March to May and subsequent ocean pr oduction from May to September in the Arctic. The ice algal production, although smaller than that of the ocean, is of ecological importance as a food source for higher trophic levels during the long arctic winter before ice melt. The simulated mean open-ocean upper 100m primary production within the Arctic Circle was $413 \pm 88\ Tg\ C\ yr^{-1}$ in the years 1998 to 2006, close to the remote sensing derived estimate of $419 \pm 33\ Tg\ C\ yr^{-1}$ but with higher interannual variations. The mean sea ice algal production in the Northern Hemisphere from 1998 to 2007 was 21.3 T$g\ C\ yr^{-1}$, which is in the range of multi-observational estimations of 9 to 73 $Tg\ C\ yr^{-1}$ based on in situ measurements. Model-data comparisons were conducted with various regional observations and the observed trend of temporal and spatial variation of the primary production. The model results compared well with the following observations and observed trends: 1) a similar increase of ocean primary production from 2003 to 2007 in the arctic open water areas as derived from remote sensing data; 2) regional annual ice and ocean primary production measured in the Bering and Chukchi seas, and Canadian Basin; 3) primary production rate with phytoplankton size composition and Chl a concentration along an arctic cruise track in the Chukchi Sea and Canadian Basin from August 2 to September 7, 2008; 4) observed decadal changes of ocean primary production from the 1990s to 2007 due to rising temperature and increased open-ocean area in the western Arctic. The changes were shown as a trend of a northward shift of production with a decrease in the Bering Sea and an increase in the arctic shelf. The in clusion of the ice-ocean ecosystem model in the physical climate model in this s tudy was successful in the simulation of the coupled ice and ocean primary prod uction in the Arctic. This will improve our estimates of deep ocean carbon expo rt and air-sea CO_2 fluxes and increase our understanding of past and future ecol ogical and biogeochemical changes in the Arctic.

MULTI-DIMENSIONAL MEASUREMENT OF PENGUIN FORAGING BEHAVIOUR USING DATA LOGGERS

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OBJECTIVES

Antarctic Peninsula region is one of the main habitats of Antarctic krill (*Euphausia superba*) and top predators such as *Pygoscelis* penguins feeding on krill. Among *Pygoscelis* penguins, Adelie (*P. adeliae*) and chinstrap (*P. antarctica*) penguin populations have decreased during the last two decades, but not in gentoo (*P. papua*) penguins. Foraging success during the breeding season is one of the most important factors that impact on the reproduction of penguins. Thus, the determination of species-specific foraging behaviour of the penguins as well as their surrounding marine environment during breeding season will provide key information about how they respond to local environmental change. In this study, we aim to determine the locations of foraging and record precise predatory behaviour of penguins, by using recently developed animal-borne data loggers.

METHODS

The study was conducted at Barton Peninsula, King George Island, South Shetland Islands (62^{o}14.30'S, 58^{o}46.50'W), where chinstrap and gentoo penguins breed sympatrically. The study period was between 4 December, 2009 and 19 February, 2010. This covered the chick-guarding period for both species. We mainly used 3 types of loggers to measure the foraging behaviour: 1) GPS loggers: determining the detailed locations on the sea (resolution<10m) every seconds as well as dive depth and water temperature (D and T), 2) camera loggers: taking pictures every 5 seconds at sea with D and T, and 3) 3-axis accelerometers: measuring 3-axis acceleration at a sampling rate 32 Hz with D and T. The accelerometers were attached simultaneously on the back and the head of penguins and the body and head movements were compared. The loggers were attached on a total of 50 chinstrap and 57 gentoo penguins. The birds were released and recaptured 1-5 days later. Besides the logger deployment, we conducted the observations on food contents delivered to the chicks by parent birds.

PRELIMINARY RESULTS DURING THE AUSTRAL SUMMER 2009-2010

GPS loggers recorded the locations of the penguins at sea (Fig. 1). Mean trip duration (±SD) was 11.6±5.6 and 10.0±3.4 (h) for chinstrap and gentoo penguins, respectively (ANOVA, p = 0.29). Mean maximum distance from the colony during the trips was 19.7±11.9 and 16.3±8.5 (km) for chinstrap and gentoo penguins (ANOVA, p = 0.31). Mean dive depth >5m was 33.2±10.9 and 38.7±13.0 (m) for chinstrap and gentoo penguins (ANOVA, p = 0.20). Among these birds with GPS loggers, stomach contents were sampled from 6 chinstrap and 7 gentoo penguins. More than 90% of the wet weight of the food contents was Antarctic krill. Samples from 3 chinstrap and 2 gentoo penguins included Antarctic Silverfish (*Pleuragramma antarcticum*). These food samples will help

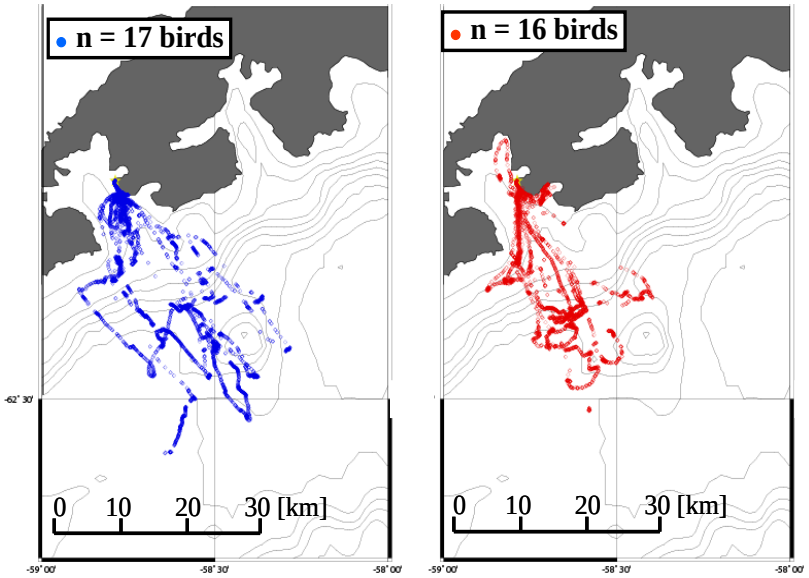

Fig. 1. *All determined locations of the chinstrap (left) and gentoo (right) penguins on the sea.*

to understand regional characteristics of Antarctic krill. Underwater images taken by camera loggers showed that most prey was Antarctic krill for both species. One gentoo penguin captured 2 large fishes, and the both penguin species sometimes encountered with chains of salps when they were ascending to the surface. 3-axis accelerometers attached on both back and head of the penguin showed that the penguins moved their head frequently presumably when they fed on Antarctic krill. Precise predation movement will be analyzed from these data. Overall, the combination of the observations using three types of data-loggers with food contents sampling will be valuable for understanding species-specific foraging ecology of the penguins and their surrounding environments in the Antarctic coastal marine ecosystem.

COMBINING SATELLITE REMOTE SENSING WITH SEA ICE MASS BALANCE STUDY

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Oban, Argyll, United Kingdom, PA37 1QA
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Arctic sea ice extent during 2007 summer plummeted to 40% below the long-term mean [1], and the second and third lowest records occurred in the summers of 2008 and 2009 [2]. This decline is occurring faster than is predicted by current climate models [3]. Climate models predict continued, and possibly accelerated, decline in Arctic sea ice extent, leading to a summer ice-free Arctic within this century [4], or even within the next thirty years [5]. The decline of Arctic sea ice is most evident in the Chukchi Borderland (CBL) region since 1995, suggesting multiple-factors contributing this rapid decline in that region. While it is a complex interplay of various factors, the key ingredients of massive sea ice loss can be related to thermodynamic melt and dynamic ice advection/breakup. This study aims at measuring these key ingredients via autonomous ice mass balance buoy (IMB) and satellite remote sensing. The key idea is to deploy two or three IMBs (i.e. SAMS IMBs) during 2010 *Araon* Arctic cruise, and to take high-resolution SAR imagery (i.e. TerraSAR-X) following the IMB-deployed floe(s) and surrounding area throughout the annual cycle. This can provide information of vertical growth/melt (via IMB), and ice advection and lateral melt/breakup process (via satellite images).

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3. Stroeve J., et al. (2007), *Geophys. Res. Lett.*, vol. 34, L09501, doi: 10.1029/2007GL029703.
4. Boe, et al. (2009) *Nature*, 2,(5), 341-343.
5. Wang, M., and J.E. Overland (2009) *Geophys. Lett*., 36, L07502, doi:10.1029/2009GL037820.

PLENARY LECTURE Ⅱ

A LANDSCAPE SCALE APPROACH TO PREDICTING BIODIVERSITY IN THE DRY VALLEYS, SOUTHERN VICTORIA LAND, ANTARCTICA.

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ABSTRACT:

For the past 50 years, terrestrial Antarctic biological research has concentrated in the Central Victoria Land region, largely targeting single terrestrial life forms such as plants, insects, or bacteria, and often carried out at diverse and unrelated locations. This compartmentalization of research disregarded the complexity of biology and resulted in difficulties in predicting the effects of various influences on structuring Antarctic terrestrial ecosystems.

A seven year duration landscape-scale Terrestrial Antarctic Biocomplexity Survey (nzTABS) has recently been undertaken initially through the support of New Zealand's IPY initiative. Our integrated and interdisciplinary approach is designed to elucidate the present biodiversity, and to predict the effects of physicochemical factors on this unique ecosystem. Three southern Dry Valleys (Miers, Marshall and Garwood) were mapped onto a mosaic of 'tiles' to create a GIS representation of the landscape. The tiles cover unique combinations of aspect, elevation, slope, and geomorphological features. 575 select tiles were targeted during'08-'09 and '09-'10 field seasons for field surveys and sampling to provide input data for the GIS model. Field surveys were conducted for vegetation and macroinvertebrates; and soil samples were collected for molecular surveys of bacteria, archaea, and fungi, microinvertebrates counts, and geochemical analyses. Meteorological stations, time-lapse cameras, and soil temp/Rh loggers have been deployed and will provide high-resolution climatological data. Finally, experiments measuring biological activity took place during the '09-'10 field season.

All collected physicochemical and biological data will be integrated into a fine-scale GIS framework, linking biodiversity, landscape, and climatological factors. The resulting ecosystem model will not only describe the distribution of key species and linkages between abiotic and biotic components, but it will also allow prediction of biological "hotspots" and the effects of climate change on this fragile ecosystem, providing information extremely valuable to policy makers. I will report on the progress of the survey and preliminary results from the recent field seasons.

SESSIONⅢ

STRESS RESPONSES OF COLD-ADAPTED ORGANISMS

IMMUNOMODULATING ACTIVITY OF POLAR LICHENS

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Lichen species were collected from King George Island (Antarctica) and were examined for their immunomodulatory effects.
(1) Among the lichens tested, the methanol extract (CR-ME) of *Caloplaca regalis* showed the highest nitric oxide (NO) production in murine peritoneal macrophages. Therefore, this study further examined the ability of *C. regalis* to induce secretory and cellular responses in macrophages. The CR-ME treatment induced tumoricidal activity and increased the production of TNF-α, and nitric oxide by macrophages. Our results indicate that the tumoricidal activity induced by CR-ME is mainly due to TNF-α and NO production, and the activation of macrophage by CR-ME is mediated probably via the p38 MAPK and pathway. These data also suggest that CR-ME might have potential therapeutic utility to treat cancer.
(2) We examined the effect of ramalin on the expression of adhesion molecules induced by TNF-α in cultured mouse VSMC line, MOVAS-1. Preincubation of VSMC with ramalin dose-dependently inhibited TNF-α-induced adhesion of THP-1 monocytic cells as well as protein expression of vascular cell adhesion molecule-1 (VCAM-1). Ramalin abrogated TNF- induced phosphorlyation of p38, ERK, and JNK. Ramalin was also shown to inhibit NK- B activation induced by TNF-α. Evidence presented in this report demonstrated that ramalin inhibited the adhesive capacity of VSMC and the TNF-α-mediated induction of VCAM-1 in VSMC by inhibiting the MAPK/NF B signaling pathway, which may explain the ability of ramalin to suppress inflammation.
(3) In conclusion, our results may provide some leads in the development of new immunomodulating drugs.

Table 1. Nitrite production from the peritoneal macrophages stimulated with the methanol extracts from eight polar lichens.

Test lichens (100 g/ml)	Nitrite (M)
None	1.43 ± 0.04
L1	2.71 ± 0.16
L2	1.97 ± 0.32
L3	1.89 ± 0.06
L5	0.53 ± 0.06
L6	2.47 ± 0.16
L8	3.15 ± 0.27
L9	1.63 ± 0.08
L17	14.63 ± 0.47*

The macrophages were treated with eight extracts for 18 h. The culture supernatants were collected and the nitrite level was measured, as described in materials and methods. The results are mean ± S.E.M of quintuplicates from one representative experiment. *: Significantly different from the control (no treatment); $p<0.05$.

Fig. 1

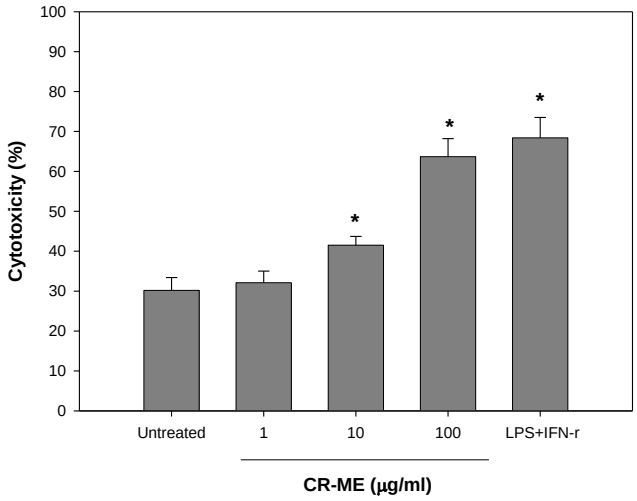
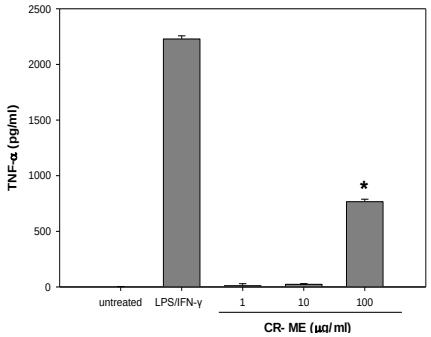


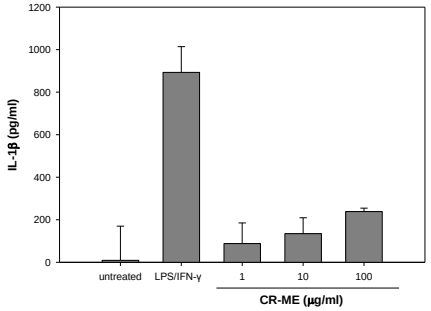
Fig. 1. *Cytotoxicity of B16 tumor cells by CR-ME-activated macrophages. The peritoneal macrophages were stimulated with various doses of methanol extract of Caloplaca regalis microalga (CR-ME) for 18 h. The macrophage tumoricidal activity was determined as described in Materials and Methods. The data shown are the results of an initial effector/target ratio of 10:1. The results are reported as a mean + S.E.M of quintuplicates from a representative experiment. As a positive control, IFN- (100 U/ml) combined with LPS (1 µg/ml) was used. *: Significantly different from the control (no treatment); p<0.05.*

Fig. 2

(A)



(B)



(C)

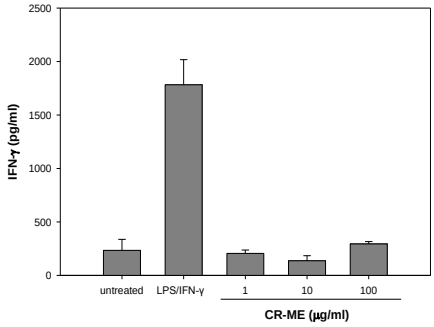
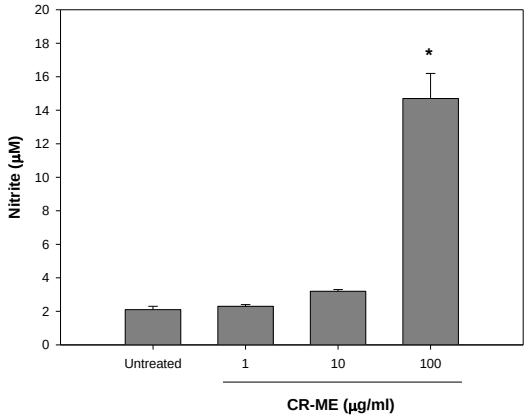


Fig. 2. *TNF- , IL-1 and IFN- production by peritoneal macrophages stimulated by CR-ME. Macrophages were treated with CR-ME for 18 h. Culture supernatants were collected, and the levels of TNF- IL-1 and IFN- were measured by ELISA, respectively. The results shown are the mean + S.E.M of quintuplicates from a representative experiment.*
**p<0.05; significantly different from control (no treatment).*

Fig. 3

(A)



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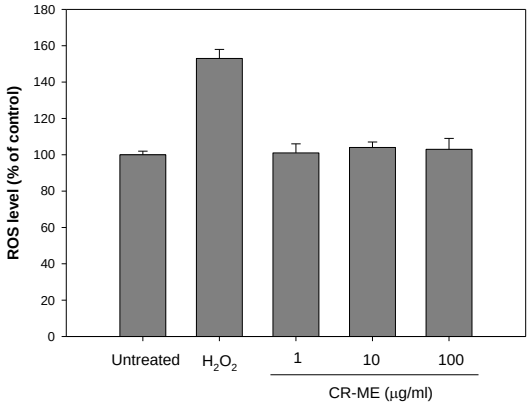
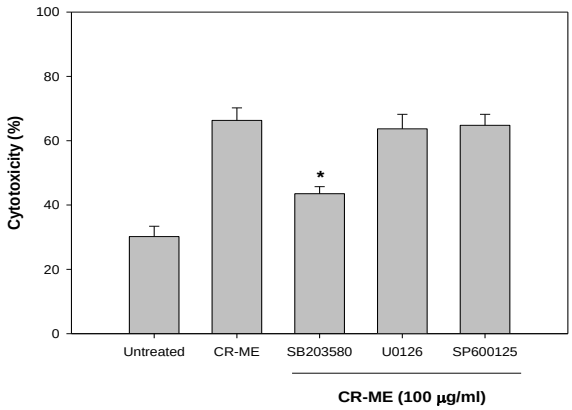


Fig. 3. *Nitrite (A) and ROS (B) production from the peritoneal macrophages stimulated with CR-ME. The macrophages were treated with CR-ME for 18 h. (A) The culture supernatants were collected and the nitrite level was measured, as described in materials and methods. (B) The macrophages were incubated either with CM-H_2-DCFDA (5 uM) for 15 min at 37 ℃ for the ROS level. Stained cells were analyzed by flow cytometry. The results are mean ± S.E.M of quintuplicates from one representative experiment. As a positive control, H_2O_2 (0.1 mM) was used. *: Significantly different from the control (no treatment); $p<0.05$.*

Fig. 4

(A)



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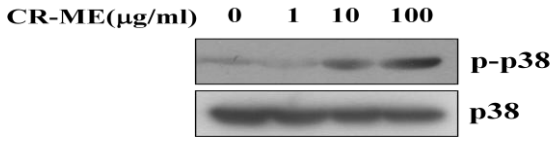

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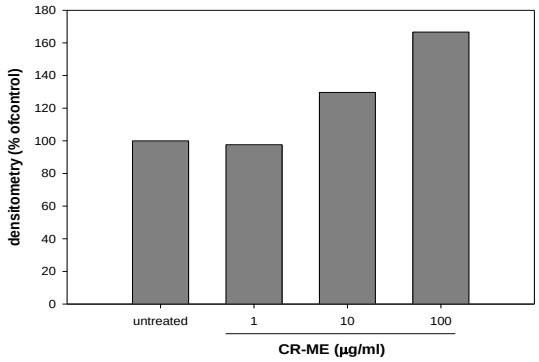

Fig. 4 *Inhibition of the CR-ME-induced tumoricidal activation of macrophage by p38 MAPK inhibitor. The peritoneal macrophages were cultured for 18 h in the medium or in the medium supplemented with CR-ME (100 μg/ml) in the presence or absence of the various inhibitors. The macrophage tumoricidal activity was determined, as described in Materials and Methods. The data shown here are the results at an initial effector/target ratio of 10:1. The results are mean ± S.E.M of quintuplicates from one representative experiment. (B) Activation of p38 MAPK in CR-ME-treated cells. The cells were treated with CR-ME (100 μg/ml) and incubated for 10 h. The whole cell lysates were prepared and used for the p-p38 or p38 MAPK Western with the respective antibodies. As a positive control, IFN- (50 U/ml) combined with LPS (1 μg/ml) was used. (C) Each value was measured by densitometric analysis of the immunoblot based on the density of the band in the untreated control as 100 %. Similar observations were obtained in three other experiments. *: Significantly different from CR-ME-treated; p<0.05.*

Fig. 5

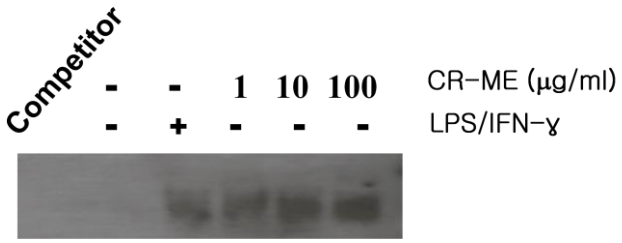

Fig. 5. *Activation of NF-κB in the CR-ME-stimulated macrophages. DNA-binding and the effects of CR-ME treatment on NF-κB DNA-binding activity in nuclear extracts of macrophages. The cells were treated with various CR-ME doses for 6 h. The cells were lysed and analyzed for their NF-κB DNA-binding activity with the use of an electrophoresis mobility shift assay, as described in Materials and Methods. The experiment was performed in triplicate with identical results. As a positive control, IFN- (50 U/ml) combined with LPS (1 μg/ml) was used.*

Fig. 6

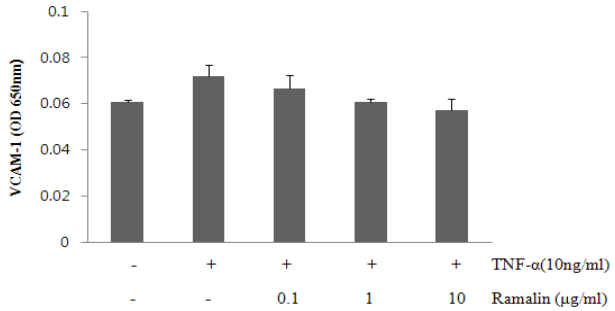

Fig. 6. *Ramalin inhibits TNF- -stimuh with the indicated concentrations of diosgenin, followed by stimulation with TNF- (10 ng/ml) for 8 h for measurement of VCAM-1. Expression of VCAM-1 in SMC after pre-incubation with diosgenin was measured by ELISA.*
The absorbance of each well was quantified in a microplate reader at 650 nm. Results shown are mean ± S.E. of one representative of three independent experiments

Fig. 7

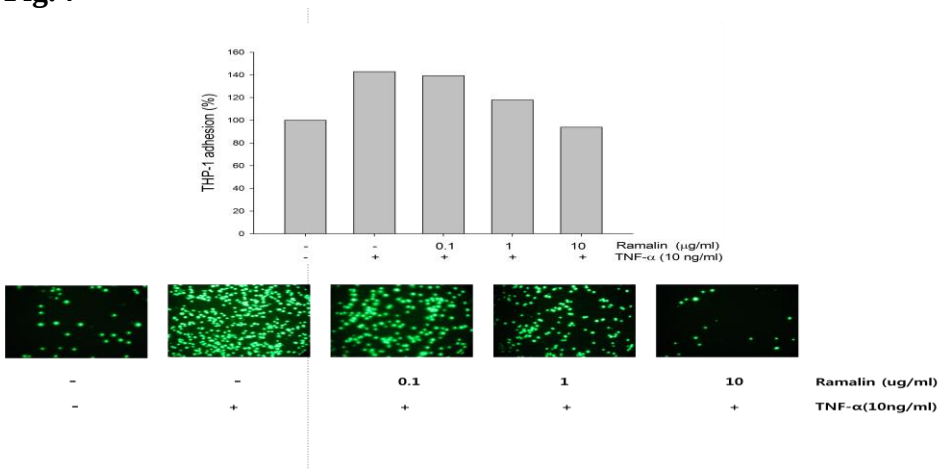

Fig.7. *Inhibition of adhesion of THP-1 cells to TNF- -stimulated SMC by diosgenin. Confluent SMC were preincubated for 2 h with the indicated concentrations of diosgenin. The cells were washed twice with medium and incubated with TNF- (10 ng/ml) for 8 h. The BCECF-labeled THP-1 cells were added to the SMC monolayer and allowed to adhere for 1 h. The results are expressed as the mean ± S.E.M. of two independent experiments performed in triplicates.*

Fig. 8

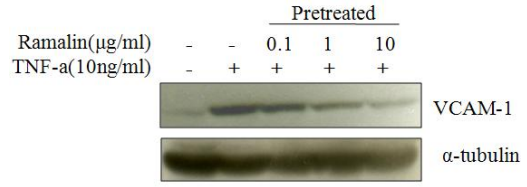

Fig. 8. *The effect of Ramalin on VCAM-1 protein expression in VSMCs. Cells were pre-treated for 2 h with indicated concentrations of Ramalin and then were stimulated by TNF-α (10 ng/㎖) for 8 h. Total cell lysates were subjected to SDS-PAGE and immunoblotted with antibodies for VCAM-1 and α-tubulin, respectively.*

Fig. 9

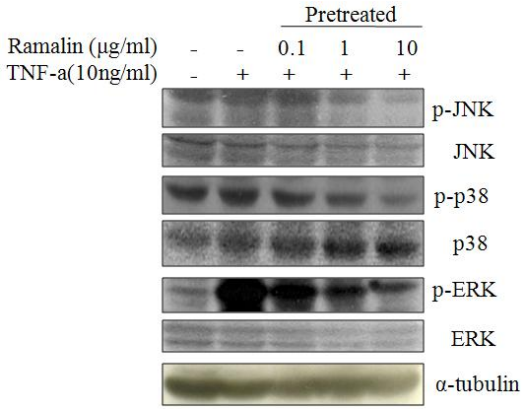

Fig. 9. *The effect of Ramalin on the phosphorylation of MAPKs by TNF-α in VSMCs. Cells were pre-treated for 2 hr with indicated concentrations of Ramalin and then stimulated by TNF-α (10 ng/ml) for 15 min. The levels of phosphorylated ERK, p38 and JNK were determined by Western blot analysis.*

Fig. 10

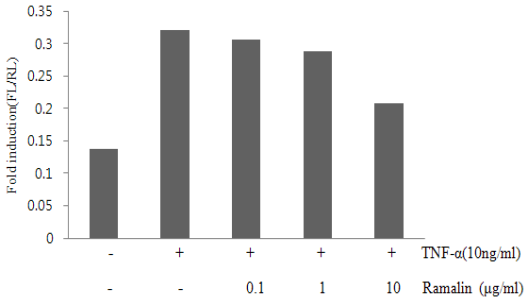

Fig. 10. *The Effect of Ramalin on TNF- -induced NF-κB activity in VSMCs. The cells were transiently transfected with NF- B-luciferase construct and were treated with indicated concentrations (0.1,1,10 μg/ml) of Ramalin for 2h, and then was treated with TNF- (10 ng/ml) for 15min. The luciferase activity was determined.*

IMMUNE RESPONSE IN THE ANTARCTIC SEA URCHIN (*STERECHINUS NEUMAYERI*): CELLULAR AND MOLECULAR CHARACTERIZATION UNDER THERMAL STRESS.

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Assessing the physiological capacities of Antarctic marine invertebrates to sub-lethal temperatures can indicate whether these organisms could adjust to new environmental conditions (Peck, 2005a). A system that could help in this adaptation is the innate immune system, present in all invertebrates, which allows protecting themselves and maintains a certain balance between different populations of microorganisms found in the marine environment or inside their bodies.

Echinoderms (particularly the purple sea urchin, *Strongylocentrotus purpuratus*) have been used extensively as a model organism in biology. This species was the first marine animal with its genome sequenced (Sea Urchin Genome Sequencing Consortium, 2006). One of the most characteristic features of its genome is that it possesses a large number and diversity of innate immunity genes, mainly at the level of receptors, a unique feature when compared with other species. It is remarkable the vast repertoire of receptors involved in processes of recognition of pathogens, such as Toll receptors, which comprises between 4% and 5% of the genes identified in the genome of the *S. purpuratus*.

The vast amount of genomic information currently available for the sea urchin model could be have a major impact in understanding the various adaptation mechanisms of Antarctic organisms to extreme conditions. Information on genome sequences from Antarctic species is limited to a certain number of fish and microorganisms (Peck *et al*., 2005b), existing only limited genomic information relevant to some Antarctic marine invertebrates. For example, there are only five sequences of *S. neumayeri* available in the GenBank database. The information regarding the immune mechanisms involved in Antarctic marine invertebrates is also limited. The few studies that exist have been carried out mainly in the context of the inflammatory and phagocytosis responses at low temperatures (Silva *et al*., 1998, 1999; Silva and Peck, 2000; Silva *et al*., 2001).

In this study we investigated the immunological processes at low temperature of the Antarctic sea urchin (*S. neumayeri*) in response to heat stress using a cellular and molecular approach. We will also use the gene information from the purple sea urchin for isolated and we will characterize the relevant immune genes of *S. neumayeri*.

Antarctic sea urchins were challenge with lipopolysaccharides (LPS) or heat killer bacteria and their cellular response was evaluated by the number and Phagocytic Capacity (PC) of coelomocytes. A significant increase in the percentage of red sphere cells was observed in sea urchins exposed to LPS for 2 ($p<0,05$) and 24 hours ($p<0,001$) (Table I). Moreover, a significant increase in the total number of cells ($p<0,001$) was observed after 2 hours in sea urchins induced by LPS. Also, a significant rise in the PC in animals stimulated by LPS was evidenced at 2 ($p<0,001$), 6 ($p<0,01$) and 24 ($p<0,05$) hours. On the other hand, the expression of the immune genes allograft inflammatory factor type-1 (AIF-1) and metallothionein (MT) decreased after stimulation with LPS for 2, 6 and 24 hours, in comparison with the control group. However, after 36 and 48 hours the gene expressions came back to normal levels. LPS could be affecting cell proliferation and modifying the immune gene expression. On the other hand, the bacterial mix increased the transcription of the AIF-1 at 24 hours.

We have cloning and characterized for the first time in Antarctic sea urchin an AIF-1. This gene participates in the inflammatory response during graft recognition, but recently has been implicated in the activation of macrophages in the immune response. The cDNA Sn-AIF-1 has a size of 608 bp and encodes a 151 aa long polypeptide. The deduced amino acid sequence has a putative size of 17.430 Da and an isolectric point of 4.92. The Antarctic sea urchin AIF-1 shows two EF hand-like motifs that usually bind calcium ions. Blast analysis revealed close matches with other known AIF-1. The deduced amino acid sequence of the Sn-AIF-1 was similar to AIF in invertebrates such as sponges, vertebrates such as fish, mouse and human. The major degree of identity between the Sn-AIF-1 and other known invertebrates was with the sponge *Suberites domuncula*, with a 38%.

We also study the short term effect of temperature on the innate immune system of Antarctic sea urchins. After exposure to different temperatures (0, 2 and 4℃) for 24 hours, the coelomocyte fluid was collected via the peritoneal membrane and cell types were evaluated and quantified. Coelomocyte fluids were evaluated with yeast for 2 hours to analyze Phagocytic Capacity (PC). We also studied the gene expression of MT and AIF-1, using actin as a housekeeping gene. Results obtained show a quantitative increase of red sphere cells at 2℃ in all periods analyzed and a decrease at 4℃, except to chronic period of 2 days, in which red sphere cells continued increasing (Fig. 1). These data are similar to those found in literature for sea urchins exposed to stress factors. Other data worth mentioning are the increasing of phagocytic capacity at 2℃ and its decreasing at 4℃; it is possible to observe that this increase coincide with the rise of red sphere cells, which suggests a possible role of these cells in the immune response. The PCR revealed a decreased of the expression of MT and AIF-1 compared to the control group.

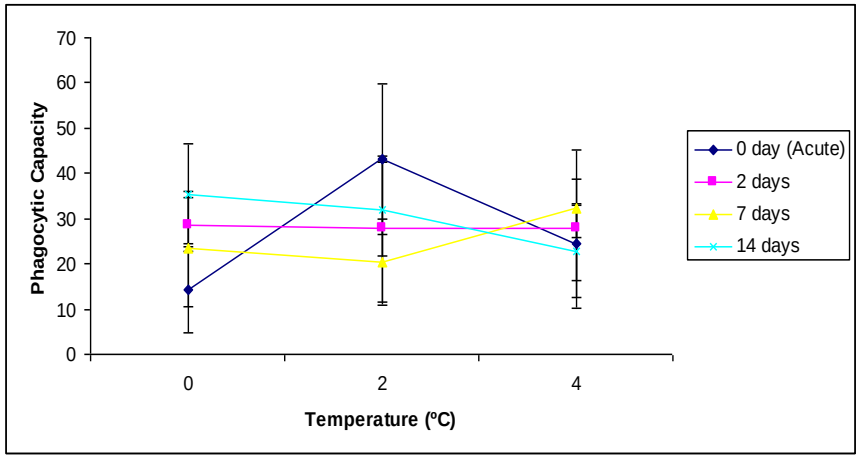
We showed that the molecular pattern of pathogens like LPS or whole bacteria could produce a modification in the number of coelomocytes and their phagocytic capacity. At the molecular level this effect was a down-regulation in the gene expression. Moreover, the down regulation gene expression related to an increased temperature may provide an opportunity to pathogens due to an impairing in the coelomocyte functions.

Table I. Number per ml of each coelomocyte type of *S. neumayeri* challenged with LPS. It is possible to evidence a significant increase in the total number of coelomocytes after 2 hours. Besides that, a significant increase of phagocytic amoebocytes, red sphere cells and vibratile cells was observed in the same peiod. PA(Phagocytic Amoebocyte), WSC (White Sphere Cell), RSC (Red Sphere Cell), VC (Vibratile Cell).

**p<α=0,05, **p<α=0,01 and ***p<α=0,001

	PA/mL (x10^5)		WSC/mL (x10^5)		RSC/mL (x10^5)		VC/mL (x10^5)		Total Coelomocytes/mL (x10^5)	
h	LPS	Control	LPS	Control	LPS	Control	LPS	Control	LPS	Control
2	20±2,6 ***	10,6±1,4 ***	4,3±1,2	3,7±1,2	32,7±3,8***	18,0±0,7***	4,8±1,3***	1,4±0,4***	3,6±0,8*	2,3±0,9*
6	17,6±2,1	14,7±1,4	3,0±0,7	1,8±0,8	24,2±3,0	21,7±1,6	1,2±0,5	1,8±0,2	2,4±0,4	3,4±1,2
24	0,62±0,3	0,5±0,4	0,1±0,4	0,1±0,01	1,0±0,8	0,8±0,1	0,2±0,3	0,03±0,01	0,1±0,03	0,15±0,03
36	8,8±1,4	9,8±0,9	1,6±0,4	1,6±0,7	12,2±1,0	14,0±2,1	0,2±0,2	0,6±0,2	1,6±0,6	2,1±0,6
48	14,1±1,4	15,6±2,9	2,6±0,4*	0,8±0,4*	20,5±2,1	20,8±2,6	1,3±0,6	1,7±0,7	2,3±0,5	2,7±1,2

Figure 1. Phagocytic capacity of the Antarctic sea urchin *S. neumayeri* exposed to different temperatures for different periods of time. A significant increase in only observed in animals exposed to 2℃ for 48 hours (acute test).



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SCREENING AND IDENTIFICATION OF PTP1B INHIBITORY ANTARCTIC SECONDARY METABOLITES FOR THE TREATMENT OF TYPE 2 DIABETES

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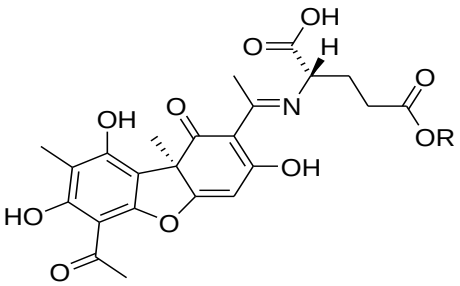
The incidence of diabetes is rapidly increasing in industrialized countries, and type 2 diabetes particularly accounts for more than 90% of cases. In type 2 diabetes, insulin-resistance is one of the characteristic types of pathogenesis, and several drugs that act by increasing the insulin sensitivity are currently being used in the clinic. However, currently available drugs for type 2 diabetes have a number of limitations, such as adverse side-effects and high rates of secondary failure.

Protein tyrosine phosphatase1B (PTP1B) is a major nontransmembrane phosphotyrosine phosphatase in human tissues and a negative regulator of the insulin-stimulated signal transduction pathway. On the basis of a number of biochemical studies demonstrating that PTP1B is a major negative regulator of insulin receptor signaling, PTP1B is now considered as an attractive target in efforts to develop new treatments for type 2 diabetes and related metabolic syndromes.

Although there have been a number of reports on the designing and development of synthetic PTP1B inhibitors, little has been studied for PTP1B inhibitors derived from natural resources. Considering the track record of success in the development of a number of useful therapeutics, it seems reasonable to search for PTP1B inhibitors from natural resources.

In the course of the searching for PTP1B inhibitors from the organisms from Antarctica, several organic extracts from Antarctic lichens and mosses were selected on the basis of their potent inhibitory effects against PTP1B. Bioassay-guided investigation on these extracts afforded several classes of compounds. The structures of PTP1B inhibitors were mainly determined by various spectroscopic analyses such as MS and NMR data, and chemical methods. Details of isolation of the active compounds and their PTP1B inhibitory activities will be presented.



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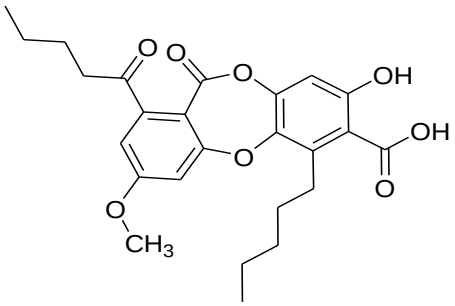
2 R = H



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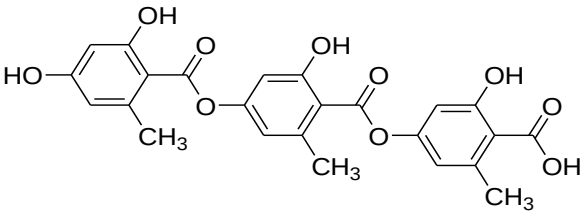

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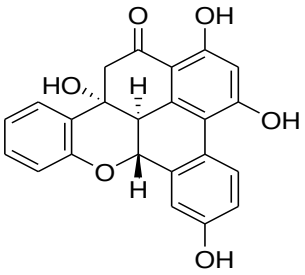

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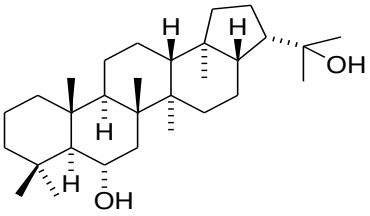

6

ANTI-DIABETIC EFFECTS OF LOBARIC ACID ISOLATED FROM ANTARCTIC LICHEN.

A-Ryeong Gwon[1], Su-Young Chae[1], Hye-Young Jeong[1], Hyungcheol Oh[2], Joung Han Yim[3], Hong Kum Lee[3], Kye Won Park[4], and Dong-Gyu Jo[1]*

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Diabetes mellitus is a chronic metabolic disorder that is caused by a failure of the body to produce insulin and/or an inability of the body to respond adequately to circulating insulin. Type 2 diabetes, previously called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, accounts for 90-95% of diagnosed diabetes cases worldwide. Type 2 diabetes is characterized by resistance to insulin, possibly due to attenuated or diminished signaling from the receptors. Pharmacological agents capable of inhibiting the negative regulator(s) of the signaling pathways are expected to potentiate the action of insulin and therefore be beneficial for the treatment of Type 2 diabetes. Protein tyrosine phosphatase 1B (PTP1B) is a major negative regulator of insulin signaling. In addition, evidence suggests that insulin action can be enhanced by the inhibition of PTP1B; however, targeting PTP1B for drug discovery is challenging because of the highly conserved and positively charged active-site pocket. The insulin-antagonizing activity of PTP1B has been demonstrated by a number of biochemical and genetic studies. For example, PTP1B knockout mice have proven to be hypersensitive to insulin and resistant to obesity, but lack other significant negative side-effects due to the mutation. This supports the inference that PTP1B is a major regulator of energy balance, insulin sensitivity, and body fat stores in vivo. Accordingly, inhibition of PTP1B is predicted to be an excellent, novel therapy to target type 2 diabetes and obesity. In this study, we evaluated anti-diabetic effects of isolated metabolite, lobaric acid from Antarctic lichen *Stereocaulon alpinum.*

COLD SHOCK PROTEINS OF POLAR MICROORGANISMS

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INTRODUCTION

At low temperatures, several challenges may threaten living organisms. Formation of stable secondary structures in DNA and RNA may inhibit transcription and translation; association of an inhibitory factor with ribosome and increase in DNA super-coiling may also hinder genetic information processing; and decreased membrane fluidity may hamper vital membrane functions such as transport and protein movement (Phadtare, 2004). Especially at the North and South Poles, temperature downshift is one of the major environmental challenges facing bacteria. Therefore, microorganisms living in the polar region require a mechanism to survive extremely cold environment

Among cold-induced proteins, cold shock proteins are the most prominent ones (Csp; Jones et al., 1987). Although Csp are widely found in various bacteria, including psychrophiles, mesophiles, and even thermophiles (Ermolenko and Makhatadze, 2002; Phadtare et al., 2003), functional studies have researched mostly Csps of a mesophilic bacterium *Escherichia coli*, but not on those of psychrophilic bacteria. In an effort to understand the molecular mechanisms of psychrophilic bacteria that allow it withstand freezing environments, we cloned eight *csp* genes from polar bacteria, and characterized some Csps in detail. Especially their ability to confer cold resistance to their hosts was evaluated.

MODEL AND EXPERIMENTS

Polar microorganisms were collected from either Antarctic coast soil (King Sejong Station, King George Island), or Arctic sea sediments (Dasan Korean Arctic Station, Ny-Alesund, Norway). They were inoculated on solid ISP4 plates (soluble starch 10 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, NaCl 1 g, $(NH_4)_2SO_4$ 2 g, $CaCO_3$ 2 g, $FeSO_4 \cdot 7H_2O$ 1 mg, $MnCl_2 \cdot 7H_2O$ 1 mg, $ZnSO_4$ 1 mg, per liter, pH 7.2), or solid Zobell plates (0.4% peptone, 0.1% yeast extract, 1.5% agar in artificial seawater prepared with 2.3% NaCl, 20 mM KCl, 5 mM $MgSO_4$ and 2 mM $NaCl_2$). The species of isolated colonies were identified by 16S rDNA sequences.

The *csp* genes were isolated using PCR amplification. A pair of degenerate PCR primers was designed from the conserved sequences of the Csp genes: CspF (5'-GGN MHC GTN AAR TGG TTY AA-3') and CspR (5'-AAR TGN ACR AAN ACR TC-3').

The *csp* sequences for homology search were obtained from the released GenBank data and aligned with the default settings of CLUSTAL W (Thompson et al., 1994).

To overproduce polar Csps in *Escherichia coli*, the *csp* gene were subcloned on pAED4 vector (Doering, 1992). *E. coli* BL21(DE3) cells transformed with the recombinant plasmids were cultured until $OD_{600} \approx 0.4$, and overproduction of recombinant protein was induced by addition of IPTG to final concentration of 0.1 mM. Overexpression of the Csp proteins were analyzed by 20% SDS-PAGE. Cells were harvested and lyzed by sonication, and Csp protein was purified by chromatography on a Q-sepharose^{TM} fast flow ion exchange column. For DNA binding assays, 2 g of purified CspA was incubated with 50 ng of biotinylated DNA probe in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 50 mM KCl, and 7.4% glycerol on ice for 20 min. The reaction products were separated on a non-denaturing 8% acrylamide gel. For *Psychromonas artica* Csps, binding ability to single-strand DNA was done using oligo(dT)-cellulose type 7 beads (Amersham Bioscience Co.) in the same buffer. To monitor DNA replication of $CspA_{St}$-overproducing *E. coli* cells, 10 μM of BrdU, a deoxynucleoside analogue, was added to the culture and incubated for 4 h at 37°C. Incorporation of BrdU into genomic DNA was measured using Cellular DNA fragmentation ELISA kit (Roche Applied Science), according to the manufacture's instruction.

The Csp protein was overexpressed in *E. coli* at 37°C for 2 h. Then, 1 ml aliquot of liquid culture was placed at -20°C for 2 h. The frozen cells were taken out of the freezer and put on ice for 1 h to be thawed. This process was repeated up to three times. Colony-forming units (CFU) were analyzed at each freeze-and-thaw cycle. The data were collected from five independent experiments.

RESULTS

Eight *csp* genes were cloned from polar bacteria isolated from Antarctic coast soil or Arctic sea sediments. They encode Csps of about 70 amino acids in length, except a Csp from KOPRI 22228. The sequence homology analysis showed that they all share similar sequences for the cold shock domain, which is implicated in nucleic acid-binding activity. The eight aromatic residues (Trp8, Phe9, Phe15, Phe17, Phe28, His30, Phe31, and Tyr39) that are critical in nucleic acid-binding by Csps (Newkirk et al. 1994) and the five hydrophobic residues (Val6, Ile18, Val27, Val29, and Val48) implicated in forming a hydrophobic core in Csps are conserved in polar bacterial Csps. Therefore, our sequence analysis suggests that they contain a typical β-barrel cold-shock domain-fold and have nucleic acid-binding activity. Indeed, in gel-shift assays with heat-denatured single-stranded DNA (ssDNA) as a probe, purified *Streptomyces* CspA ($CspA_{St}$) retarded migration of the probe band. *Psychromonas artica* Csps also bound to oligo(dT)-cellulose beads, suggesting ssDNA-binding activity of these Csps.

DNA synthesis of $CspA_{St}$ overproducing cells was monitored by incorporation of BrdU (5-bromo-2′-deoxyuridine; a deoxynucleoside analogue) into the genomic DNA. $CspA_{St}$-overproducing cells incorporated BrdU less than 5% than the control cells did, suggesting that DNA replication is severely impaired in these cells (Fig. 1).

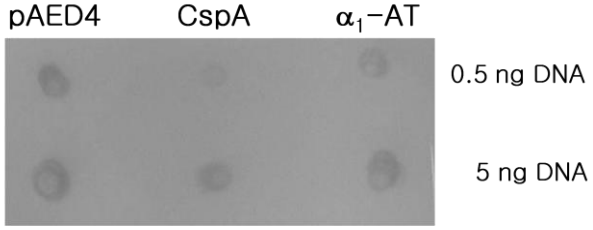

Fig. 1. *Impaired incorporation of BrdU into DNA in $CspA_{St}$-overexpressing cells.*

Interestingly, overexpression of polar bacterial Csps in *E. coli* significantly improved the freeze-tolerance of the host cells. Overexpression of most Csps increased freeze-tolerance of the hosts about 10 fold (Fig. 2), meanwhile the freeze-resistance of α_1-antitrypsin–overproducing cells (data not shown) or the vector pAED4-carrying cells were indistinguishable from that of the untransformed *E. coli* cells. Surprisingly, overexpression of CspB from KOPRI 22228 increased freeze- tolerance of the host up to 1,000 fold. The cold-sensitive phenotype of the *E. coli* quadruple *csp* deletion strain (Δc*spA*, Δ*cspB*, Δ*cspG*, Δ*cspE*), BX04, was also suppressed by overexpression of Csps from KOPRI 22228.

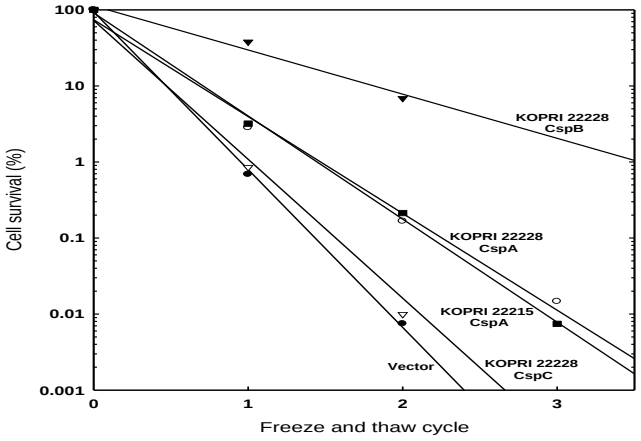

Fig. 2. *Freeze-survival ratios of polar bacterial Csp-overexpressing E. coli cells.*

SUMMARY AND CONCLUSIONS

We have cloned eight *csp* genes from polar microorganisms. The sequence homology analysis of polar bacterial Csps showed that they share significant homologies with other bacterial Csps for the cold shock domain, suggesting similar functions for Csps. DNA binding assays such as gel shift assays and co-elution experiments, showed that they indeed bind to single-stranded, but not double-stranded, DNA. When Csps from polar microorganisms were over-expressed in *E. coli*, it temporary caused cell growth-retardation and morphological elongation. Incorporation of a deoxynucleoside analogue, 5-bromo-2′-deoxyuridine, into newly synthesized DNA was also drastically diminished in $CspA_{St}$-overexpressing cells. These results suggest that $CspA_{St}$ play a role in inhibition of DNA replication during cold-adaptation. Most of all, overexpression of polar Csps drastically increased the freeze-resistance of *E. coli* strain by more than ten times, suggesting that these proteins aid survival in polar environments.

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COLOR TUNING OF MICROBIAL RHODOPSINS ON THE SURFACE OF ARCTIC AND ANTARCTIC OCEAN

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Rhodopsin is seven-transmembrane light-harvest protein that has *all-trans* retinal as a chromophore. Proteorhodopsin (PR) is discovered from marine bacteria and has a proton pumping activity from inside to outside of the cell using solar energy. Generally, PR is classified into two groups by the maximum absorption spectra. One is the BPR which absorbs blue (490 nm) wavelength and the other is the GPR which absorbs green (530 nm) wavelength region of visible spectrum. Previously, we have isolated and characterized 18 GPR homologs from the sea near Svalbard, Norway. We have confirmed the positive-correlation between proton pumping function and photochemical reaction rate. Interestingly, in the case of several variants which the residue was changed 200th TYR residue to ASN, the rhodopsin has a mild temperature sensitivity to the rate of photocycle between GPR and BPR. Based on this result we were predicted that the rhodopsins are adapted to the change of temperature in cold ocean environment. In this study, we isolated the conserved regions of rhodopsin by PCR from the sea near King George Island and compared with Arctic microbial rhodopsins. We also isolated a single full sequence (BPR type) of Antarctic rhodopsin and several sequence between helix C and F by conserved primers. We are now characterizing new Arctic and Antarctic rhodopsins and also trying to express in *E. coli* UT5600 to characterize the photochemical properties.

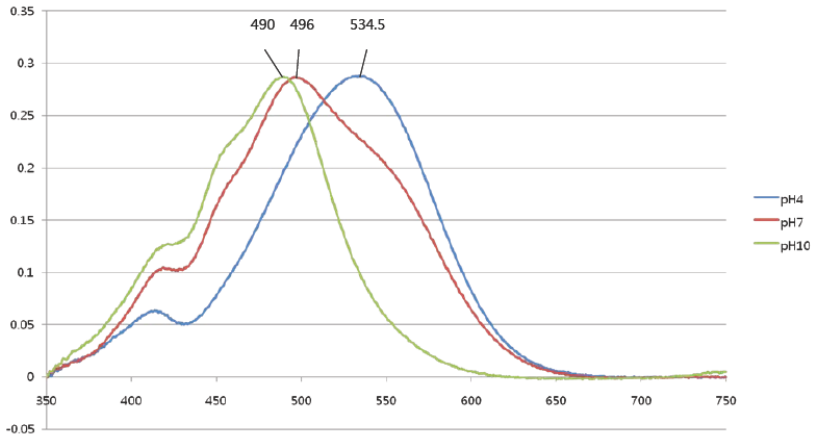

Fig. 1. *Absorption spectra for Antarctic rhodopsin on various pH*

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SESSIONⅣ

POLAR TERRESTRIAL AND MARINE ECOSYSTEMS

<TO BE ADDRESSED>

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ADAPTATION MECHANISMS OF LAKE BAIKAL MICROORGANISMS TO EXTREME ENVIRONMENTS, THEIR TAXONOMIC DIVERSITY AND BIOLOGICAL POTENTIAL

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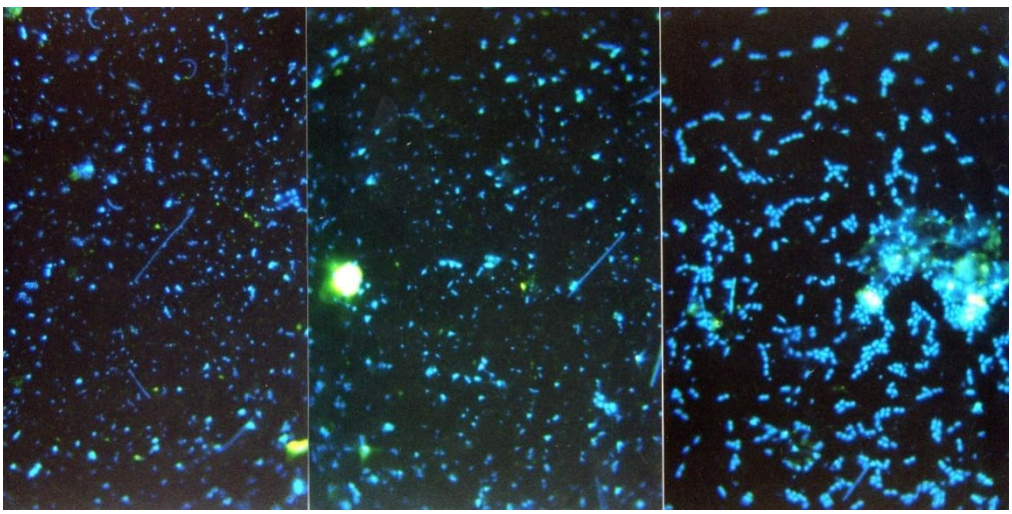
Interest to microorganisms living at low temperatures has been significantly increased for the past few years. It is obvious that they can serve as important objects for studying the most comprehensive aspects of cellular and molecular biology. Moreover, they widen our understanding about the diversity of physiological and biochemical mechanisms used by living organisms. Average water temperature in Lake Baikal varies from 2℃ to 10 ℃ (3.2 ℃ on average). Taking into account average annual temperature of the environment, it is possible to suggest that a great abundance of psychrophilic microorganisms inhabits the autochthonous microflora of Lake Baikal. Morphological diversity of these microorganisms is high (Fig. 1). They inhabit the entire multimeter water column of the lake in their viable state, their number being decreased from 100 m up to the bottom. They may be detected in a form of biofilms in the water column both solitary and in aggregates, their ability to form biofilms increasing with the depth.



A B C

Fig. 1. *Morphological diversity of Lake Baikal microorganisms: A – surface, B – 50 m, C – 100 m.*

The analysis of literature data and the results of our own studies showed that bacteria are able to transfer into unculturable state retaining their viability. This property of bacteria makes it difficult to detect bacteria in the water during traditional cultivation on diagnostic media. The cells in the Baikal water are preserved at low temperatures due to their denser and thicker walls. This helps them survive under extreme conditions. Therefore, the majority of bacteria occur in the lake water in unculturable state. It is necessary to apply methods of molecular analysis for identifying the true taxonomic diversity of these organisms.

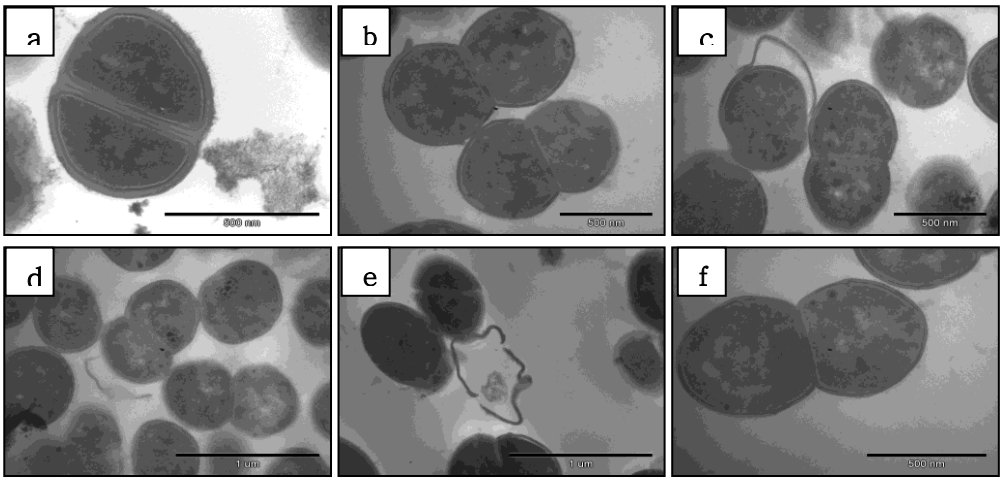


Fig. 2. *Electronic micrographs (TEM) of enterococcus cells: a – cell in unculturable state; b-e - enterococcus cells after resuscitation; f – dividing cell after resuscitation.*

Studies of species composition demonstrated a great taxonomic variety of both culturable and unculturable bacteria. The following bacterial genera and actinomycete genera dominated in the microbial community of Lake Baikal: *Pseudomonas, Bacillus, Flavobacterium, Acinetobacter, Alcaligenes, Arthrobacter, Micrococcus, Nocardia, Planococcus, Corinebacterium, Xanthomonas, Zoogloea, Caulobacter, Micromonospora*, and *Streptomyces*. Molecular-biological methods proved the presence of microorganisms of these genera in the lake water (Bel'kova, 2003; Parfenova, 2006).

Representatives of the genera *Pseudomonas* and *Bacillus* have high biochemical activity with regard to organic compounds (phthalates, polycyclic aromatic hydrocarbons) and are able to produce at low temperature protease, lipase, lecithinase, amylase, acid and alkaline phosphatases. Actinomycetes and flavobacteria actively produced antimicrobial substances.

Baikal strains are active producers of biologically active substances and can be used for production of new prospect antibacterial drugs. Their ability to produce active exoenzymes gives us a possibility to successfully use extremophilic microorganisms for practical purpose.

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MICROBIAL DIVERSITY IN LAKE KHUVSGUL AND LAKE UVS, MONGOLIA AND IN FRESHWATER SPONGE OF LAKE BAIKAL, RUSSIA

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To scrutinize the microbial diversity of Mongolian and Russian lakes, we investigated structure and characteristic of bacterial community in two Mongolian Lakes (Khuvsgul and Uvs), and in fresh water sponge of Lake Baikal, Russia. In Mongolian lakes, bacteria were isolated with R2A medium and their enzyme activities were characterized. The isolates were identified based on their 16S rRNA gene sequences. And water samples were analyzed by DGGE for identifying community structure including unculturable bacteria. The bacterial communities in Baikal sponges and ambient water near the sponge were analyzed with above same methods. *Acidovorax defluvii* , *Sphingobacterium faecium* , *Flavobacterium succinicans*, *Mycoplana bullata* and *Acidovorax. Facilis*, isolated from Lake Khuvsgul, were identified and Gamma proteobacterium, *Pseudomonas* sp., *Vibrio vulnificus*, *Stappia aggregata IAM 12614*, *Pseudomonas mendocina ymp*, *Dickeya dadantii Ech703*, *Rheinheimera pacifica* were from Lake Uvs. Bacterial enzyme activities from Uvs were higher than those of Lake Khuvsgul. Moreover, bacterial community was more diverse in Lake Uvs than that of Lake Khuvsgul. And marine bacteria such as *Vibrio vilfinicus*, *Stappia aggregate* IAM 12614 and *Rheinheimera pacifica* were islolated from Lake Uvs, where salinity is about 0.9%. In Lake Khuvsgul, there was bacterial stratification. In surface layer, *Acidovorax defluvi* was obtained, and 2 m depth, *Fluavobacterium succinicans* was dominant, and at 5 m depth, *Mycopalana bullata* was dominant, but at 10 m and 25 m depths, *Acidovorax facilis* was dominant. In Lake Uvs, we could not find out depth discrimination, but number of DGGE band was 11, which is more diver than that of Lake Khuvsgul (number of band=7). The 92 strains of oligotrophic and psychrotrophic bacterial were isolated from two sponges and ambient water near the sponge from Lake Baikal, Russia. Thirty five bacterial strains are isolated from ambient water near the sponge, 27 bacterial strains from *Baikalospongia* sp., 30 bacterial strains from *Lubomirskia* sp.. The 16S rRNA sequence analysis of selected 31 bacterial strains was carried out. By these results, 18 strains belong to *Pseudomonas* sp.. Other strains, 3 strains belong to *Acinetobacter* sp., 3 strains to *Yersinia ruckeri*, 3 strains to *Bacillus* sp., 2 strains to *Paenibacillus* sp. and *Brevibacterium* sp., *Buttiauxella agrestis*. By DGGE, the numbers of band from ambient water near the sponge and 2 sponge species (*Baikalospongia* sp. and *Lubomirskia* sp.) were 12, 10 and 11, respectively. Bacteria in ambient water near the sponge were more diverse than bacteria in 2 sponge species. The common dominant species of three samples was *cyanobacterium*. Totally 102 clones were randomly selected from two clone libraries constructed with DNA fragments from Baikalian sponges. *Cyanobacteria*-like cluster was occupying 32% and 40% of total sequences

from two sponges (*Baikalospongia* sp. and *Lubomirskia* sp.) respectively. Twenty one percent of the clones were matched with the sequences of *Actinobacteria*, which are releasing bioactive materials such as antibiotics. Sponge is functioning primary producer, as symbiotic relationship with bacteria in Lake Baikal. In Mongolia, there are many numbers of saline lakes where bacterial community is diverse. As bacteria are living as parasites and/or symbionts, there would be many new species in Mongolian saline lakes. Also there are many numbers of clean and oligotrophic fresh water lakes which contain a lot of bacterial diversity. By this reason, Mongolian and Siberian lakes are treasury of microorganisms.

STUDIES IN SECONDARY PRODUCTION – COPEPOD FEEDING

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Copepods are probably the most abundant marine metazoans and constitute >90% of the total zooplankton abundance in several parts of the world's oceans. Planktonic copepods are key organisms in aquatic ecosystems, because they are the most important link between the primary productivity of microalgae and the production of many fish species in the oceans. The quantification of phytoplankton utilization by grazing allows to calculate the downward transport of organic matter. Phytoplankter are the primary processors of photoautotrophically synthesized organic matter in the oceans, and zooplankton plays major roles in the nutrient transfer to higher trophic levels. Quantifying rates of phytoplankton utilization by copepods is a necessary step to understand the mechanisms that regulate phytoplankton populations in marine ecosystems and the flux of organic matter. However, different zooplankton taxa play different roles with different impact, also in the polar environment. Copepod feeding processes cannot easily be generalized, as copepods exhibit herbivorous, omnivorous, carnivorous and detritivorous feeding habits. Quantifying rates of phytoplankton utilization by copepods is a necessary step to understand the mechanisms that regulate phytoplankton populations in marine ecosystems and the flux of organic matter. We aim to investigate the ingestion, gut content, evacuation, and clearance rates of copepods belonging to various ecological and phylogenetic groups, that are collected at the sea ice water interface and from the water column. Our objective is to understand the feeding ecology of copepods in the Arctic at different spatial (horizontal and vertical) and temporal scales (particularly, diurnal with a fine resolution of hourly intervals, and season). Gut pigment contents of copepod species will be correlated with the ambient environment (chlorophyll a concentration, seawater temperature, illumination by Pearson correlation).

FEEDING ECOLOGY OF DOMINANT GROUNDFISH IN THE NORTHERN BERING SEA DETERMINED BY STOMACH CONTENTS AND STABLE ISOTOPE ANALYSIS

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BACKGROUND:

The Bering Sea is one of the most productive areas in the sub-Arctic region including commercial fisheries in the southeastern Bering Sea, and it is also an important foraging area for seabirds and mammals (Loughlin et al. 1999, Aydin & Mueter 2007). With the ongoing, but variable climate change influence on the reduction of sea ice extent and coincident increases in seawater temperatures, it is likely that the Arctic biological community will be affected (Overland & Stabeno 2004, Grebmeier et al. 2006, Serreze et al. 2007, Mueter & Litzow 2008). The benthic dominated ecosystem on this shallow northern Bering shelf may well transition to a more pelagic-dominated system over time as sea ice and zooplankton grazing patterns change with continued climate warming (Grebmeier et al. 2006b, Grebmeier & Barry 2007). Ultimately trophic structure and coincident energy pathways of dominant organisms would also likely change.

To examine the diet of dominant groundfish communities in the northern Bering Sea, stomach contents were used. Stable isotope analyses were also used to strengthen food web studies, since the stable isotope abundances of carbon (C) and nitrogen (N) in tissues are determined in part by the isotopic content in the diet (Fry & Sherr 1984, Michener & Schell 1994).

METHODS:

Most of the fish used in this study were collected in the northern Bering Sea around St. Lawrence Island (SLI) during two cruises on the USCGC *Healy* in spring 2006 and 2007 using otter and beam trawls. Additional groundfish samples were collected during a survey supported by the Norton Sound Economic Development Corporation summer 2006 on R/V *Pandalus*, and on a T/S *Oshoro-Maru* research cruise

summer 2007. Benthic invertebrate and zooplankton samples were collected in the study area too.

Prey in the fish stomachs were identified to the lowest possible taxonomic level. In this study, graphical approach of Amundsen et al. (1996) was used for stomach contents to explore prey importance, feeding strategy and niche width. Diet overlap also was evaluated using Schoener's index.

Fish and prey samples for stable carbon and nitrogen isotopes were oven-dried and pulverized to a fine powder and were analyzed on a Thermo – Electron Delta plus XL isotope ratio mass spectrometer (IRMS). The trophic level (TL) of individual organisms was estimated using the following equation: $TL = 2 + (\delta 15N_{consumer} - 8.2) / 3.8$.

Statistical tests were performed with NCSS 2007 software.

RESULTS & CONCLUSIONS:

Arctic cod (*Boreogadus saida*), Bering flounder (*Hippoglossoides robustus*), snailfish (Liparidae), Arctic staghorn sculpin (*Gymnocanthus tricuspis*), Shorthorn sculpin (*Myoxocephalus scorpius*), and Arctic alligatorfish (*Ulcina olrikii*) were dominant groundfish within the bottom trawl survey. Of the 297 fish stomachs examined, all of Bering flounder were empty in 2006 and 2007. All other fish species contained prey in their stomachs.

In summary, our results indicate that benthic amphipods, particularly ampeliscid amphipods, are the most important prey for the dominant groundfish in the northern Bering Sea, except shorthorn sculpin. Ampharetid polychaetes were preferred prey by two sculpin species. Shorthorn sculpin and Arctic alligatorfish also consumed crabs. The only occasional planktonic feeder was Arctic cod which preyed on calanoid copepods and euphausiids in addition to benthic amphipods. Although some of these dominant fish species share the same habitat and food resources, no strong evidence of competition is found.

Stable isotope results in this study are consistent with stomach content analyses undertaken on the same fish samples. The mean $\delta 13C'$ values for groundfish were correlated to the estimated TL using $\delta 15N$ values and to the increase of $\delta 13C$ per TL which was 1.8‰.

Arctic cod consume higher proportions of benthic amphipods in the Bering Sea, while copepods are their main prey in both the Chukchi or Beaufort seas. Therefore, Arctic cod from this study have higher TL values than fish from the Arctic Ocean proper.

$\delta 15N$ enrichment of benthic invertebrates from the base food source was less in this study than other areas. Generally, regions of high pelagic-benthic coupling result in short food webs and higher benthic production, while areas of less export of carbon to the benthos results in longer food chains and lower benthic production (Grebmeier et al. 1989, Grebmeier & Dunton 2000, Dunton et al. 2006).

The results of our study indicated that the combination of stable isotopic data with prey content analyses provide for a stronger data set to interpret the changes in $\delta 13C$ or $\delta 15N$ values within the food web. However, our study helps convey the complexity of stable

isotopic variability because the stable isotope analyses alone cannot explain whether differences are caused by food source availability or stable isotope variation in prey due to environmental factors (Fry et al. 2008 and references therein). Thus, our work emphasizes the importance of combined use of fish stomach contents and predator-prey isotopic analyses to provide more insights into food web relationships of fish-prey populations.

MICROBIOLOGICAL ANALYSIS OF EXTREMOPHILOUS COMMUNITIES OF ANTARCTICA, THE ARCTIC ZONE AND LAKE BAIKAL

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BACKGROUND.

Microbiological analyses of cultivable and uncultivable microbial community inhabiting cold extremal environments have been carried out.

MATERIALS AND METHODS.

We studied: 1) Ice cores of the boreholes 5G, VK-07 and VFL from archives of station Vostok (Antarctica) taken in 2006-2007, 2) Ice cores collected in the vicinity of Berezovy Cape (Southern Baikal) in March-April, 2007-2008, 3) Water and sediments samples taken on the 13th stations of the shelf of Kara Sea (2009).

Sampling preparation to the analyses was realized keeping to the terms of the sterility at all steps. Methods of fluorescence hybridization in situ (FISH), of light, epifluorescence and scanning electron microscopy as well as cultivation (direct plating and plating from storage culture) on nutrient media with different composition have been used.

RESULTS & CONCLUSIONS

It was found that the total bacterial abundance (TBA) in Antarctica ice cores was higher (100-180 thousands cells/ml) at the period of maximum graciation (440-442 m, ~22 thousand years), but it was on one order less in warm periods (194-195 m, ~7,5 thousand years и 1823-1824 m, ~126,6 thousand years) and averaged 40-50 thousands cells/ml. According FISH data the TBA changed from 100 to 300 thousands cells/ml and was presented by groups of Eubacteria that made up the highest TBA percentage – 31-41%, whereas representatives of the kingdom Archea were in smaller number – 0.5-1.2%. The majority of bacteria belonged to γ- and α-subgroups of Proteobacteria. Aggregated microorganisms were observed in significant abundance in some samples of ice core the morphological diversity of which were identical to some bacteria of a rod-shaped form and rarely to a round form of different sizes. Chains of rods and rods with tails of a *Caulobacter* type were also found. Scanning microscopy also was shown an abundance of bacterial-mineral structures and microorganisms of unusual morphology. Morphological diversity of cultivable microorganisms is represented by spore-forming and non-spore-forming bacteria, as well as by actinomycetes, yeast and fungi. Microorganisms were revealed in 89% of samples under direct plating and in 95% - under preliminary cultivation. Maximum number of heterotrophic bacteria arranged 25-

65 CFU/ml in Holocene and in Interglacial period. Maximum number of oligotrophic bacteria (38-62 CFU/ml) was found in the same periods. Psychrophilic bacteria were found in 28% of samples during direct plating and in 51% - during preliminary cultivation. Bacteria isolated from the ice corns had high enzyme activity. From 245 tested strains 48% of cultures possessed protease activity, 40% - phosphatase, 58% - lipase, 62% - amylase, 20% dilute collagen and 32% of the strains decomposed lecithin. Seventy two strains that were able to degrade proteins, lipids and carbohydrates and twenty six strains producing protease, lipase and amylase as well as alkaline phosphatase have been displayed.

Discovery of cryophilic communities in the ultrapure ice cover of Lake Baikal (Obolkina et al., 2000) was a certain surprise to the limnologists. It has been proven that ice communities were one of the keystone elements of Lake Baikal ecosystem. The bacterial ice community of Lake Baikal was represented by small mostly short rods and cocci (0.3 to 1.5 μm in diameter), either as free-living cells organisms or as aggregated long strands and shapeless colonies, or found in the crustacean pellets. In March 2007, large amounts of yeast-like cells and actinomycete mycelium were found in the middle ice layers of the cores collected at the site 50 m from the coast near Berezovy Cape. In 2007, the TBA varied from 63 to 220 x 10^3 cells ml^{-1} in the ice cores collected in the vicinity of Berezovy Cape and the highest concentration was observed in the lower layers of 60.5 cm and 63 cm long cores. In 2008, quantitative analyses of bacteria collected at the sites 50 and 290 m from Berezovy Cape showed a drop in their numbers in comparison to the preceding year, the abundance being 200 thousand cells/ml. In 2007, bacterial concentration varied from 17 up to 220 thousands cells/ml in the ice from a deep water part of the lake. Their highest abundance in the lower layers of a 65 cm long core was 212 thousand cells/ml and 220 thousand cells/ml in a 68 cm core. The middle layers exhibited an increased TBA compared to the over- and underlying layers. None of analogous tendencies in the distribution of bacteria in the ice cores from a deepwater site were reported in 2008. Phylogenetic diversity were represented by groups of Eubacteria (60-90% of TBA), Archea (2,5-3,3%), α- and γ-Proteobacteria (25-43%) and β-Proteobacteria. Morphological diversity of cultivable microorganisms was represented by Gram-positive and Gram-negative rods and cocci, spore-forming bacteria, yeast and actinomycetes.

The maximal total bacterial abundance in the surface water of the shelf Kara Sea reached up to 515 thousands cells/ml and in the bottommost layers – up to 549 thousands cells/ml. Psychrophilic bacteria growing in 4-6^{0}C dominated in the cultivable microbial community. Their number was in four times higher them the number of organotrophic bacteria in water and in sediment samples. The number of psychrophilic bacteria depended on temperature conditions of the region, the great quality of the organic matter inputting with river flows and as a result of sea flow and outflow.

The work was supported by the Program of RAS ONZ-7.13 – project 7.13.7, Program of Presidium RAS № 20 project 20.7 and by Interdisciplinary project SB RAS № 96.

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POSTER SESSION

COMPARATIVE STUDY OF MICROBIAL RHODOPSINS BETWEEN ARCTIC AND ANTARCTIC OCEAN

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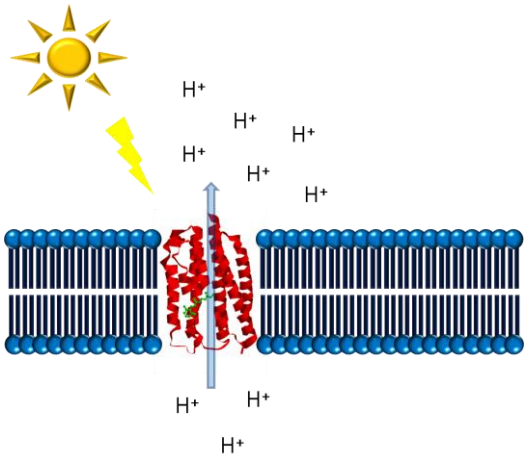

fig 1. *diagram of PR as light-harvesting proton pumping protein*

Rhodopsin is seven-transmembrane light-harvest protein that has all-trans retinal as a chromophore. Proteo-rhodopsin (PR) is discovered from marine bacteria and has a proton pumping activity from inside to outside of the cell using light energy. Generally, PR is classified into two groups by the maximum absorption spectra. One is the BPR which absorbs blue (490 nm) wavelength and the other is the GPR which absorbs green (530 nm) wavelength region of visible spectrum. Previously, we have isolated and characterized 18 GPR homologs from the sea near Svalbard, Norway. We have confirmed the positive-correlation between proton pumping function and photochemical reaction rate. And we obtained several rhodopsin variants in the position of 200th residue. In the case of GPR type rhodopsin, Tyr200 is conserved and Asn200 is found in BPR type rhodopsin. Our Tyr200Asn variant indicated a mild temperature sensitivity to the rate of photocycle betseen GPR and BPR. Based on this result we were predicted that the rhodopsins are adapted to the change of temperature in cold ocean environment.

In this study, we isolated the several conserved regions of rhodopsin like gene by PCR from the sea near King George Island and compared them with Arctic microbial rhodopsins. And we also found several sequence between helix C and helix F of rhodopsin by conserved primers. We also isolated two of full sequence of Antarctic rhodopsin. One was exactly same sequence with MBP (GPR type) and another one was a newly discovered sequence. We tried to determine the photochemical properties of newly found rhodopsin protein. It was indicated that it absorbs blue region, about 500nm wavelength and it is able to pump protons out through cell membrane. Now we are doing measure the photoconversion rate of newly founded rhodopsin. And we are going to found the full length sequence of several small fragments (above-mentioned) through discover the N-terminal and C-terminal sequence of by AP-PCR. And then we will characterize the spectral properties and photochemical properties of discovered new rhodopsin proteins.

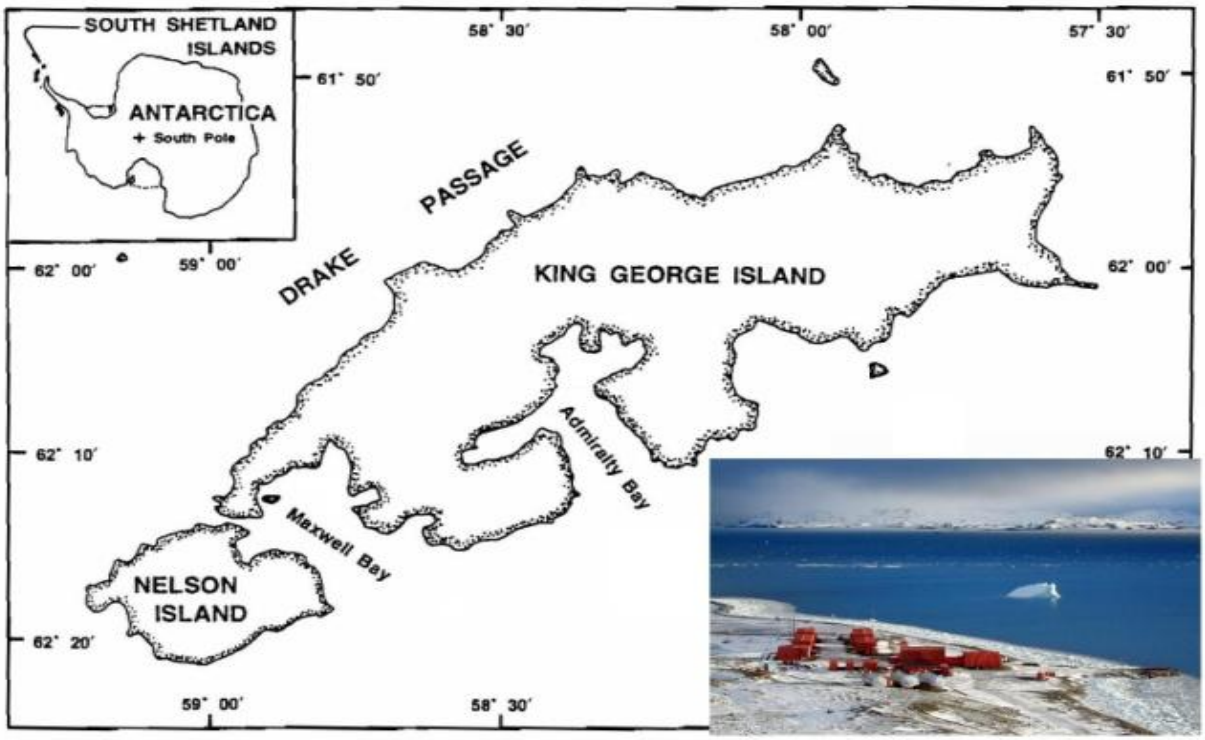

fig 2. *The map of Antarctic King Sejong Station (62˚ 13′ S, 58˚ 47′ W) and the whole view (http://www.kopri.re.kr)*

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TWO MAIN ITALIAN SCIENTIFIC INSTITUTIONS CARRIED OUT SEVERAL MARINE ENVIRONMENTAL CAMPAIGNS FROM 1996 UP TO NOW, IN THE REGION AROUND THE SVALBARD ISLANDS AND IN THE BARENTS SEA:

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- the ENEA-Marine Environment Research Centre, La Spezia, through the participation in a) EU-funded multinational programmes "ARMARA",1996-1999 and "REMOTRANS", 2000-2003 (www.santeresa.enea.it/Artico/home.htm); b) the programme "CABANERA", funded by the Norwegian Research Council, 2003-2006,and c) through bilateral collaboration between ENEA and the Polish IOPAS, 2006-2008;
- the CNR- Institute of Marine Science, La Spezia, through the national "Strategic Arctic Programme", 2000-2003, and the on-going new "Climate Change Integrated Project". The CNR is actually managing the Italian Station "Dirigibile Italia" in Ny Ålesund (www.dca.cnr.it).

The main results obtained by these multy-year and multy-disciplinary programmes will be briefly illustrated, in the field of:

- Sedimentation processes occurring in a glaciated Arctic fjord (Kongsfjord), with focus on the glacier/sea interface,
- The oceanographic features of the inner Kongsfjord.
- The fluxes of carbon to the sediments of the Barents Sea;
- The *long-range* dispersion of natural and anthropogenic radionuclides in the region.

KOPRIA LITUS GEN. NOV., SP. NOV., OF THE FAMILY *OXALOBACTERACEAE*, ISOLATED FROM ANTARCTIC COASTAL SEAWATER

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ABSTRACT

A Gram-negative, non-motile, catalase- and oxidase- positive, strictly aerobic and short-rod-shaped bacterium that was designated strain KOPRI 25157^T was isolated from coastal seawater sample in around King Sejong Station, in King George Island, Antarctica. The temperature and pH ranges for growth on R2A agar were 10-20℃, and 5.0-10.0, respectively. Phylogenetic analyses of the 16S rRNA gene sequence of strain KOPRI 25157^T have showed that it belongs to the family *Oxalobacteraceae* of the class *Betaproteobacteria*, but it formed a distinct clade from other recognised members of the family. Major ubiquinone was Q-8. Predominant cellular fatty acids were $C_{10:0}$ (0.2%), $C_{10:0}$ 3OH (3.2%), $C_{12:0}$ (2.9%), $C_{16:1}$ $\omega7c$/15 iso 2OH (56.4%), $C_{16:0}$ (30.5%), $C_{18:1}$ $\omega7c$ (3.7%), $C_{18:0}$ (0.7%), and 11 methyl $C_{18:1}$ $\omega7c$ (2.4%). On the basis of these data, it is proposed that strain KOPRI 25157^T is the representative of a novel genus, named *Kopria* gen. nov. is proposed in the family *Oxalobacteriaceae.* The type strain for *Kopria litus* sp. nov. is KOPRI 25157^T (=JCM 16673^T =KCTC 23040^T).

CARBON AND NITROGEN ISOTOPE RATIOS IN THE ANTARCTIC LIMPET NACELLA CONCINNA FROM ROCKY COASTAL HABITATS, MARIAN COVE, KING GEORGE ISLAND

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ABSTRACT

The Antarctic limpet *Nacella concinna* successfully colonizes intertidal and subtidal rocky shores and tide pools of Marian Cove, King George Island, Antarctica in the austral summer. $\delta^{13}C$ and $\delta^{15}N$ of the limpet tissues and their potential food sources were measured to determine their dietary origins and their movements between diverse habitats. $\delta^{13}C$ and $\delta^{15}N$ of the organic matter sources of epilithic microalgae, macroalgae, and suspended particulate organic matter (SPOM) were readily distinguishable to discern their relative contribution to the limpet diets, with the most depleted values being found in SPOM and the most enriched in macroalgae. The limpets exhibited a spatial trend in distribution due to their seasonal migration, with smaller individuals in the subtidal zone as compared with larger ones on the intertidal sites. The limpet isotopes had relatively broad ranges of $\delta^{13}C$ and $\delta^{15}N$ (−26.6 to −12.8‰ and 2.6 to 7.1‰, respectively), suggesting a dietary shift between habitats as well as size classes. The stable isotope ratios for each habitat seem likely to reflect the differing availabilities of the three potential food sources. Isotope mixing model results indicate a spatial shift in dietary mixture between habitats as well as limpet size classes. Epilithic microalgae and phytoplankton made great contributions to the diet of the subtidal limpets. Together with epilithic microalgae, macroalgae were significant contributors to the intertidal limpets where macroalgae were abundant. A higher contribution of macroalgae to the limpet diets was found in the tide pools. In contrast, while phytoplankton was an important food source for the limpet spats, a great dietary dependence on epilithic microalgae was found in the small-size limpets from the lower intertidal zone. Our results suggest that limpet grazing (i.e., top-down control) can determine microalgal and/or macroalgal abundance and coverage on the Antarctic rocky-shore ecosystem, and trophic structure of benthic food web can change along environmental gradients even at spatial scales of dozens or hundreds of meters in the Antarctic.

INTRODUCTION

The patellid limpet *Nacella concinna* (Strebel 1908) is one of the most common macrobenthic invertebrates colonizing the Antarctic and sub-Antarctic rocky shores (Picken 1980; Davenport 1988). The Antarctic limpet, which is a benthic grazer, displays the most important biomass of intertidal invertebrates in King George Island (Fraser 1989). This limpet resource represents an important dietary component to the energy requirements of Antarctic kelp gulls (Fraser 1989; Favero et al. 1997). Limpets may, therefore, play a crucial role as a trophic mediator between primary producers and predators in the Antarctic rocky-shore food web.

Growth performance of the Antarctic limpets decreases towards higher latitudes (Clarke et al. 2004). Although their annual growth rate is relatively low, their seasonal growth rate and condition are maximized in the austral summer (December–February). Spawning occurs during the time when both the water temperature and the food availability are increased (Brêthes et al. 1994; Kim 2001). These authors demonstrate a direct relationship between the somatic and gonadal mass of the limpets and the abundance of microphytobenthos. In particular, Kim (2001) found that no clear spawning events occurred in the limpet population of the King George Island coast during an austral summer when filamentous algae and benthic diatoms were poorly developed in the spring.

In the present study, we investigated the stable isotope ratios of carbon and nitrogen in the dominant organic matter sources of epilithic microalgae, macroalgae, and phytoplankton, together with the limpet tissues in Marian Cove, King George Island, Antarctica. The Antarctic limpets successfully colonize the intertidal and subtidal rocky bottoms and the tide pools during the austral summer. Tide pools are rocky pools that are filled with seawater even at low tide on the intertidal zone. The tide pools are unique habitats, which are separated from exposed intertidal habitats only at low tide, and colonized by various flora and fauna. High densities of the Antarctic limpets also can be found within the tide pools. The aim of the present work was to determine the food sources of the limpet populations and to examine their dietary shift according to site conditions among different habitats.

RESULTS AND DISCUSSION

A dual isotope plot of $\delta^{13}C$ versus $\delta^{15}N$ for the potential food sources and the consumers enables us to interpret the food sources assimilated (Fig. 1). The $\delta^{13}C$ values of the limpet spats were very close to those of SPOM as shown by the suspension feeder *L. elliptica*. The limpet $\delta^{13}C$ values from the subtidal B zone, the intertidal W, and the smaller-size creatures from the intertidal B zone were aligned with those of epilithic microalgae. However, the values of the limpets commonly collected in the intertidal B and tide pools were shifted towards those of macroalgae and positioned between those of epilithic microalgae and macroalgae.

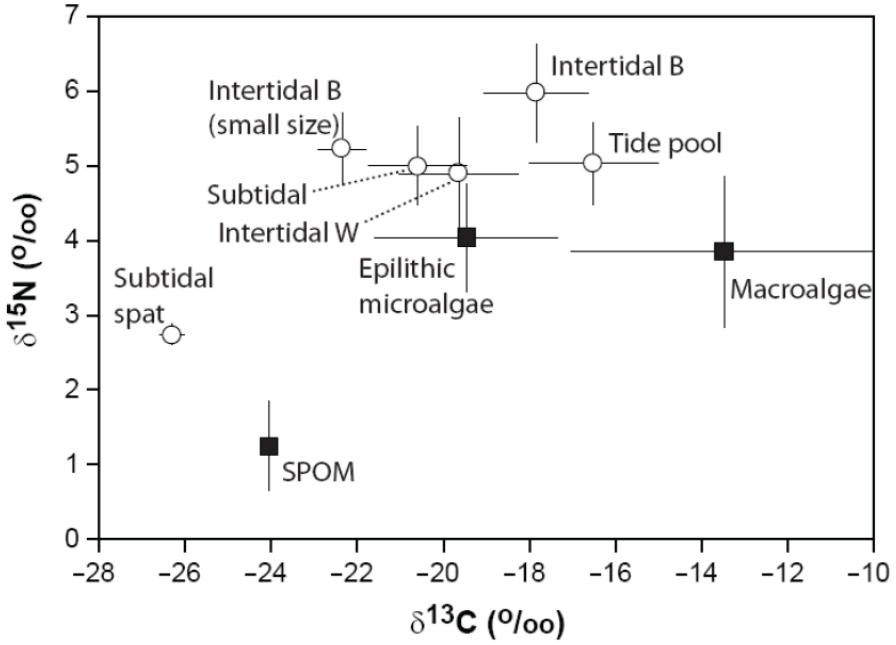

Fig 1. *Dual plot of $\delta^{13}C$ and $\delta^{15}N$ values of organic matter sources (square) and the limpets (circle) collected in different habitats of King George Island in February 2008.*

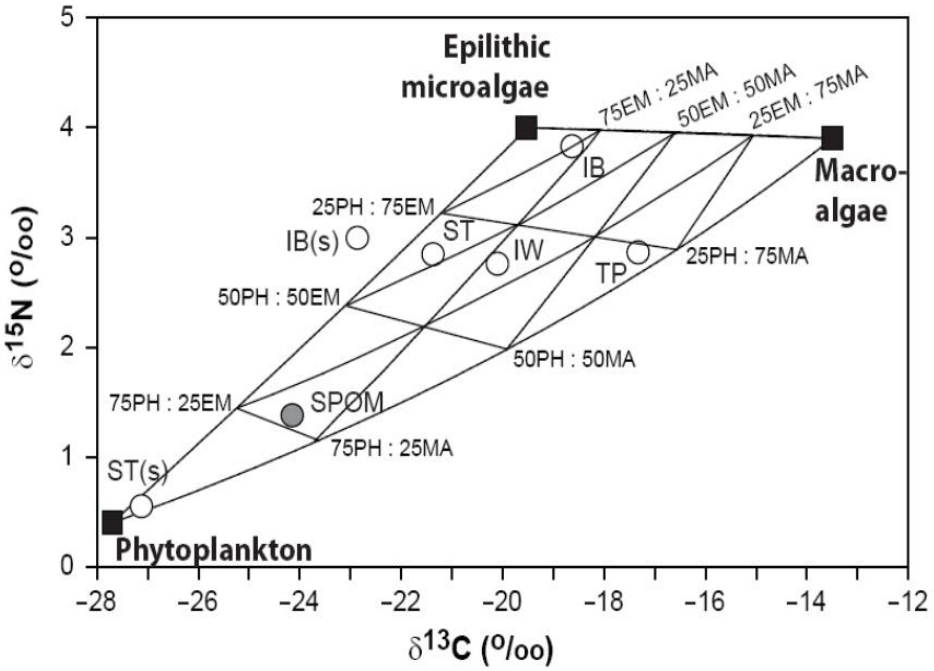

Fig 2. *Mixing triangle for the concentration-weighted model. Variations in percentage contribution of phytoplankton (PH), epilithic microalgae (EM), and macroalgae (MA) are shown along the edges of the mixing triangles.*

Based on trophic fractionation of +0.8‰ and +2.2‰ for limpet-tissue δ13C and δ15N from the diet, δ13C and δ15N plots for the presumed diet of the limpets showed considerable shifts among habitats (Fig. 2). Therefore, three-source mixing model suggested spatial variation in relative contribution of primary organic matter sources to the limpet diet. The result indicated that epilithic microalgae and phytoplankton made

great contributions (54% and 36%, respectively) to the diet of the subtidal limpets, with a minor contribution of 10% by macroalgae. Along with the high contribution (72%) of epilithic microalgae, macroalgae may be a significant contributor (22%) to the large-sized limpet diet of the intertidal B zone. A higher contribution (67%) of macroalgae to the limpet diets was found in the tide pool site, with a considerable contribution (28%) of epilithic microalgae. Epilithic microalgae and phytoplankton made nearly equal contribution (about 37%) to the diet of the limpets was detected on the intertidal W, with an important contribution (27%) of macroalgae. On the other hand, while epilithic microalgae occupied the major part (83%) of the diet of the small-sized limpets with a shell length of 10.7–19.0 mm collected in the lower part of the intertidal B, the contribution of macroalgae was negatively estimated. In contrast, phytoplankton were the only sources contributing to the diet of the limpet spats with a shell length of 3–5 mm collected at the subtidal zone. Model estimation also suggested that phytoplankton primarily occupied SPOM. However, epilithic microalgae and macroalgal debris also constituted considerable parts (10 and 16%, respectively) of SPOM.

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FLOW CYTOMETRIC OBSERVATION OF PICOPLANKTON COMMUNITY STRUCTURE IN THE RUSSIAN CHUKCHI SEA IN 2009

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ABSTRACT

We investigated the relationship between the spatial structure of hydrographic properties of different water masses and the variability of different picoplankton groups to discern the factors controlling the distribution of picoplankton in different parts of the Chukchi Sea using a flow cytometry. It is very important to study the Chukchi Sea as an only gateway between the North Pacific Ocean and the Arctic Ocean in order to understand arctic marine ecosystems responding to the current climate changes, but there have been not much study because of the difficulties in logistics. The RUSALCA cruise in 2009 provided very important opportunities to research marine environments and ecosystems in the Russian and US sides of the Chukchi Sea. *Prochlorococcus* and *Synechococcus* found first in the Chukchi Sea contributed about 30% in the cell abundance of small phytoplankton community (< 20 μm). In general, *Synechococcus* was more abundant in the southern part whereas *Prochlorococcus* was more abundant in the northern part of the study area in the Chukchi Sea in 2009. In contrast, eukaryotes such as pico- and nano-phytoplankton contributed about 70 % of the communities. Based on the data obtained by the flow cytometry, we found that eukaryotic phytoplankton constitute the bulk of the small photosynthethic community in the Chukchi Sea.

INTRODUCTION

The picoplankton (<2 ㎛) includes by definition the autotrophic cyanobacteria *Synechococcus* spp. and *Prochlorococcus* spp., small eukaryotic algal gropus, and heterotrophic bacteria, which are important components of marine plankton communities. These phytoplankton gropus, together with picoeukaryotes, have fast growth rates matched by high mortality losses caused by microzooplankton grazing, making them fundamental components of the biomass and primary production of marine ecosystems, and participating in nutrient regeneration and cycling in the ocean. The quatification of picoplanktonic organisms is therefore of great importance for the characterization of marine ecosystems and for understanding the function of marine food webs. *Synechococcus* and *Prochlorococcus* are easily discriminated by flow cytometry due to their different pigment compositions and size.

The Bering Sea supports high rates of primary production and extraordinary rich marine resources, which are valuable fisheries, and immense populations of marine birds and mammals. Recently, major changes in Bering Sea stocks of phytoplankton, zooplankton, and commercially important ground fish, as well as marine mammals and seabirds have correlated with temporal shifts in environmental characteristics and physical forcing mechanisms (Livingston et al. 1999). Schell (2000) hypothesized that there has been a 30-40 % decrease in seasonal primary productivity in the northern Bering Sea over several decades. However, there has been no research to support his hypothesis for the decline in the productivity in this region since the ISHTAR and BERPAC programs in the late 1980s. The aim of this study was to better understand the relationship between the spatial structure of hydrographic properties of different water masses and the variability of different picoplankton groups, and to discern the factors controlling the distribution of picoplankton in different parts of Chukchi Sea using a flow cytometry.

RESULTS AND DISCUSSION

Prochlorococcus and *Synechococcus* found first in the Chukchi Sea contributed about 30% in the cell abundance of small phytoplankton community (< 20 μm). In general, *Synechococcus* was more abundant in the southern part whereas *Prochlorococcus* was more abundant in the northern part of the study area in the Chukchi Sea in 2009. In contrast, eukaryotes such as pico and nano phytoplankton contributed about 70 % of the community (Fig. 2). Integrated chlorophyll-a concentrations were relatively low (< 100 mg chl-a m^{-2}) in the Chukchi Sea in 2009. Generally, the highest concentrations were in the central southern part of the Chukchi Sea, but these values in 2009 were much lower than those measured in previous studies. The average of integrated chlorophyll-a concentrations was 57.7 mg chl-*a* m^{-2} (± 37.8 mg chl-a m^{-2}), which was 3 times lower than that (155.6 mg chl-*a* m^{-2}) in 2004 (Lee et al. 2007).

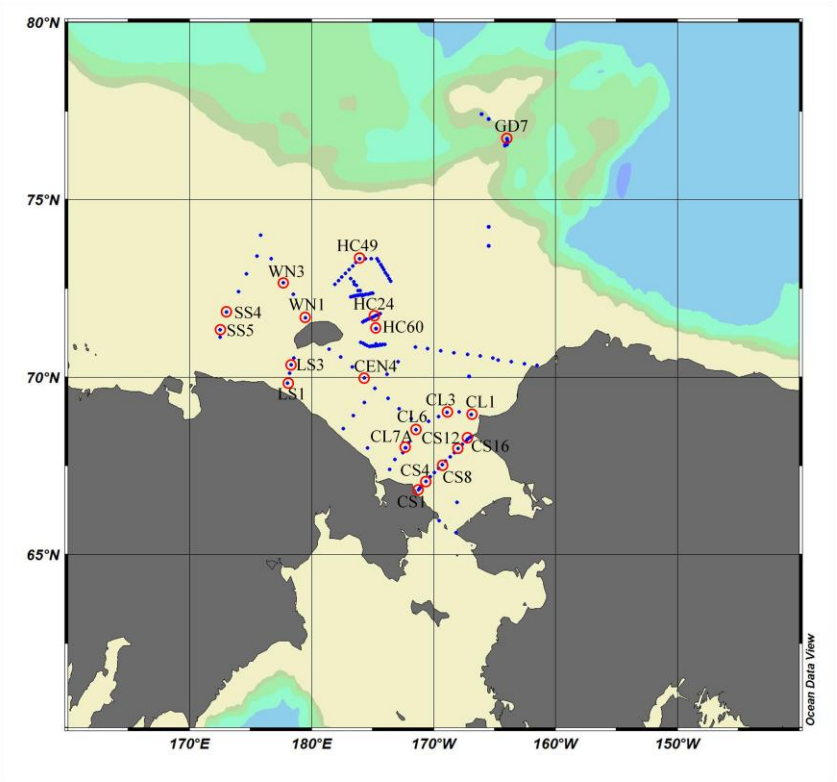

Fig 1. *Sampling stations during the Rusalca cruise in 2009*

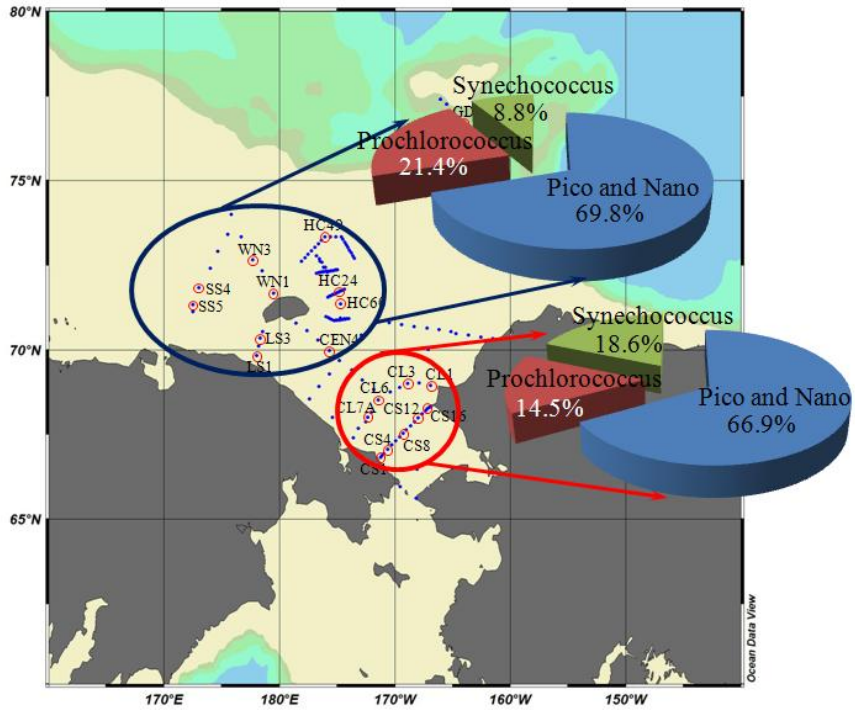

Fig 2. *Compositions of Synechococcus and Prochlorococcus*

Table 1. *Contribution (%) of Prochlorococcus and Synechococcus at the three different depths*

light	Pico-and nano-eukaryotes		*Prochlorococcus*		*Synechoccus*	
depth	Southern	Northern	Southern	Northern	Southern Northern	
100%	66.85	69.81	14.55	21.39	18.61	8.8
30%	66.06	69.94	15.58	21.31	18.36 8.76	
1%	67.74	64.04	19.13	25.56	13.14 10.4	

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CHARACTERIZATION OF COLD-ACTIVE B-*N*-ACETYLGLUCOSAMINIDASE FROM ARCTIC BACTERIUM *PSEUDOALTEROMONAS ISSACHENKONII* KOPRI 22718

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One hundred thirty-six marine bacteria showing chitinolytic activity were isolated from 47 sediment samples of the Kara Sea, Arctic. Among them, a psychrotolerant strain (KOPRI 22718) was selected for its high exo-acting chitinase activity. The 16S rRNA sequence identifies KOPRI 22718 as *Pseudoalteromonas issachenkonii*. An exo-acting chitinase was homogeneously purified from the culture supernatant of KOPRI 22718 through ion exchange and gel filtration chromatography, and the molecular weight of purified chitinase was estimated to be approximately 112 kDa. Due to its β-*N*-acetylglucosaminidase activity, W-Chi22718 was able to produce *N*-acetyl-D-glucosamine monomers from chitin oligosaccharide substrates. W-Chi22718 displayed chitinase activity from 0-37℃ (optimal temperature of 30℃), and maintained activity from pH 6.0-9.0 (optimal pH of 7.6). Interestingly, W-Chi22718 exhibited a relative activity of 13 and 35% of maximal activity at 0 and 10℃, respectively, which is comparable to the activities of previously characterized, cold-adapted, bacterial chitinases. W-Chi22718 activity was enhanced by K^+, Ca^{2+}, and Fe^{2+}, but completely inhibited by $Cu^{2+}$and SDS. We found that W-Chi22718 can produce much more *N*-acetyl-D-glucosamines from colloidal chitin, working together with, previously characterized, cold-active endochitinase W-Chi21702.

UVR EFFECTS ON BIOTA WITH EMPHASIS ON POLAR REGIONS

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Comparative measurements indicate continued increases in solar UV radiation (UVR) on land and in the aquatic environment where oceans cover 70% of the earth's surface. Ozone-related increases in solar UVR during the last decades provide an important ecological stressor with global impacts, particularly so in polar regions. All plant, animal and microbial life appears to be susceptible to UVR to a highly variable extend that depends on the individual organism and the combination with other factors of its environment. In addition, are UVR impacts masked by large seasonal and geographical differences. Exposure to UVR has been found to affect DNA, to increase mutation-rate, to impair photosynthesis, enzyme activity and nitrogen incorporation, to bleach cellular pigments and to inhibit motility and orientation, to affect reproduction and development, and reduce the productivity in several marine organisms. Hence, UVR shows effects and responses on various integration levels: from genetics, physiology, biology, populations, communities, to functional changes like in the food web with consequences on carbon flow through whole ecosystems. This way UVR affects circulations of materials and energy globally. UVR effects are well studied in marine primary producers, such as microalgae and seaweeds – with balanced publication records between temperate and polar regions. UVR effects on marine consumers like invertebrates are much less studied, particularly so in the polar environment. Even at current levels, solar UVR may provide changes of the abundance and distribution of invertebrates, particularly through impediments on critical phases of their development (e.g. early life history stages: gametes, zygotes and larvae). Although UVR is effective on patterns and processes beyond the individual, we expect molecular, proteomic and genomic, mechanisms of action and protection processes towards UVR as being fundamentally important. Differences in sensitivity between biota may relate to efficiency differences of their protection mechanisms and repair systems (e.g. the existence of DNA damage checkpoints and/or the induction of shock responses). UVR is predicted to remain elevated for the next several decades, and there will be a need to further investigate the molecular targets of UVR. This holds particularly for polar oceans where marine biota are understudied. A better understanding of invertebrate UVR protection will also allow the development of technologies for the indication and against the adverse impacts of enhanced UVR. Direct evaluation of UVR-induced DNA damage turned out to be a sensitive indicator of both, toxic and latent effects, the latter may not appear until later stages of ontogeny or in subsequent generations. UVR-

induced DNA damage is commonly estimated by quantifying CPDs (Cyclobutylpyrimidine dimmers). Less specific endpoints of genotoxicity should be considered for other forms of UVR damage. This is especially relevant since several recent studies have emphasized that both, UV-B and UV-A contribute to UVR toxicity.

LOW-TEMPERATURE PROPAGATION OF AN ISOGENIC ARCTIC CYANOBACTERIUM FOR A GENETIC SOURCE OF CONSISTENT BIOMASS PRODUCTION

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A cyanobacterium strain, *Nodularia spumigena* KNUA005 was collected near Dasan Station in Ny-Å lesund, Svalbard, and isogenically purified. An axenic culture was obtained by three purification steps: centrifugation, antibiotics treatment and streaking. The broad antibacterial spectrum of imipenem and kanamycin may have killed a wide range of heterotrophic bacteria while *N. spumigena* KNUA005 remained unaffected. This cyanobacterium showed active growth at mid to low temperatures (15~20℃) indicating the isolate was psychrotolerant. We are currently conducting experiments to incorporate the cold-resistance genes into domestic cyanobacterial strains for low-temperature production (winter season) of algal biomass.

INTRODUCTION

For large-scale open-pond algal cultivation systems usually suffer from temperature limitation in cold climate (Sheehan *et al*. 1998, Benemann 2008). Hence, polar cyanobacteria offer an interesting potential for year-round production of biomass and biofuels due to their psychrotrophic characteristics (Tang *et al*. 1997). The objective of the current study was to axenically produce cyanobacteria from an Arctic freshwater bloom sample and to test the isolate's cold-tolerance capability. Genetic isolation of cold-resistant genes will be followed for introduction into an indigenous fast-growing cyanobacterial strain.

MATERIALS AND METHODS

Sample collection. Freshwater samples were collected from the temporal water runoff region, approximately 10 km east of Dasan Korean Arctic Research Station in August 2009.

Axenic culture production. Well-grown cyanobacterial cultures were streaked onto BG-11 agar supplemented with imipenem, kanamycin and cycloheximide. Plates were placed in the dark for 24 hrs and incubated in a light:dark cycle (16:8 hrs) at 15℃. Emerging filaments were aseptically transferred to fresh BG-11 plates.

Morphological and molecular identification. Cells were inspected at X 400 magnification on a light microscope equipped with DIC optics. The PC-IGS region, 16S rRNA and region RuBisCO rbcLX were used as phylogenetic markers Neilan *et al*. 1995, Nubel *et al*. 1997, Rudi *et al*. 1998).

Cold-tolerance test. N. spumigena KNUA005 was inoculated into both BG-11_0 and BG-11(+) media and incubated in a light:dark cycle (16:8 hrs) at 10, 15, 20 and 25℃ for 14 days. The optical density (OD) was measured spectrophotometrically at 750 nm.

RESULTS AND DISCUSSION

The combination of two antibiotics with different mechanisms of action successfully prevented bacterial growth. The method used in this study may provide an effective way of axenic culture production for filamentous cyanobacteria from heavily contaminated environmental samples.

Molecular characterization showed that this Arctic cyanobacterium was *Nodularia spumigena* (over 97% sequence similarities). Its morphological features (trichomes, vegetative cells and heterocysts) also suggested that the isolate was *N. spumigena* (Fig. 1.).

As shown in Fig. 2A., *N. spumigena* KNUA005's optimal growth temperature in BG-11_0 was 20℃ and it was also able to grow well at 15℃ and 25℃. It showed a tendency of growing slower in BG-11(+) medium than in nitrogen-free BG-11_0 medium (Fig. 2B). There was little or no cyanobacterial growth in both BG-11_0 and BG-11(+) media at 10℃, but the cyanobacterium remained alive and subsequently grew well when placed under favourable conditions (data not shown). As the isolate exhibited a tolerance to low temperatures ranging 10~15℃, it is suggested that there were cold-tolerance genes present in this Arctic cyanobacterium. Hence, screening and identification of these useful genes are needed using DEG tag profiling and Solexa® massive parallel sequencing approach. Our research group has obtained four potential candidates for biofuels production isolated from cyanobacterial bloom samples in Lake Daecheong, South Korea in late summer 2009 (unpublished data). In the next research stages, cold-tolerance genes will be incorporated into these indigenous cyanobacteria for sustainable production of algae-based biofuels under unfavourable weather conditions.

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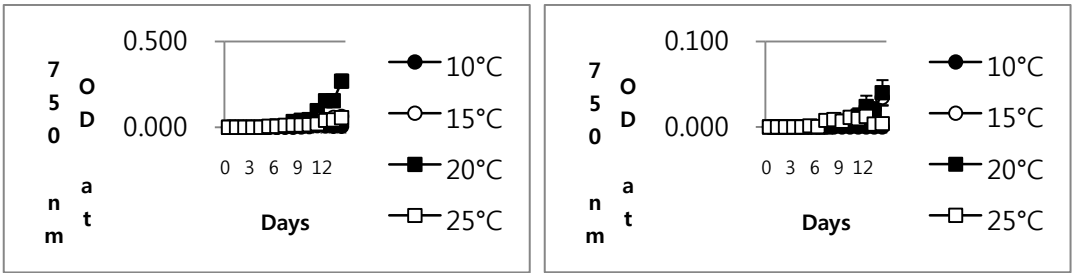
Fig. 1. *Light microscope image of Nodularia spumigena KNUA005.*



(A) (B)

Fig. 2. *Growth curves of Nodularia spumigena KNUA005 grown in BG-11$_0$ (A) and BG-11(+) (B) media. Error bars were standard deviations calculated from triplicate.*

PYROSEQUENCE ANALYSIS OF THE ANTARCTIC HAIRGRASS DESCHAMPSIA ANTARCTICA UNDER VARIOUS ABIOTIC STRESSES

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Deschampsia antarctica is the only monocot that thrives in the Antarctic region. Despite it is an invaluable resource for the identification of genes associated with tolerance to various environmental pressures, little transcriptome information is available. In order to get a broad view of genetic responses to various abiotic stresses, we synthesized and sequenced cDNA fragments from RNAs of one control and three plant samples under low temperature, high salt, and PEG treatment. Using massively parallel pyrosequencing, we obtained 370,169 *D. antarctica* expressed sequence tags (ESTs) at an average size of 212 bp per read. A majority of sequences (337,581 reads) could be assembled into 28,177 contigs with an average size of 425 bp. Finally, we could have a total of 60,765 unigenes containing 28,177 singletons. Using similarity searches against several public databases, we constructed a functional classification of the ESTs into categories such as genes related to responses to stimuli, as well as photosynthesis and metabolism. *In silico* analysis using 28,177 contigs revealed that 585, 586, and 774 contigs were specifically up-regulated, and 36, 379, and 217 contigs were specifically down-regulated by low temperature, high salt, and PEG treatment, respectively. Real-time PCR analysis of various stress responsive genes revealed different patterns of regulation in the different environments, suggesting that these genes are involved in responses to specific environmental factors

DIVERSITY OF POLYKETIDE SYNTHASE GENES IN LICHEN CLADONIA SPP.

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Lichens are well known to produce a great variety of secondary metabolites including polyketides which have diverse biological roles and potential uses in pharmaceutical application. To attain a comprehensive understanding of polyketide synthase (PKS) gene diversity of lichen species from polar regions, thirty-two *Cladonia* samples were selected from Chile, the Artic and the Antarctic regions. The β-ketosynthase (KS) domains of putative PKS genes were amplified and sequenced using degenerate primers. We obtained 25 KS sequence fragments from direct sequencing and 22 fragments from cloning. Phylogenetic analyses of 47 KS sequences have shown that 10 distinct PKS types were retrieved, of which all belonged to non-reducing (NR) PKS. Three and six types were included in NR clade I and clade II, respectively. The last type of PKS was related to NR type I and type II, but the specific relationship was not resolved well. The most abundant PKS type consisted with 25 sequences from 24 samples and the second most abundant type contained 10 sequences from 10 samples.The sample with the most diverse PKS types was included *Cladonia furcata*. Tests of hypotheses between KS domains and their ITS-26S phylogeny showed that the relationships were not significantly different in the most OTUs belonging to type 9 and type 10.

GENE EXPRESSION PROFILING OF COLD STRESS RESPONSES USING EXPRESSED SEQUENCE TAG (EST) FROM A FRESHWATER GREEN ALGA, SPIROGYRA VARIANS

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Spirogyra varians (Hass.) Kuetzing is freshwater green alga that could survive wide range of temperature (0℃-25℃). Expressed Sequence Tags (ESTs) from warm (20℃) and cold (4℃) cultivated *S. varians* were compared simultaneously to isolate cold regulated genes. A total of 5,450 ESTs from warm and cold libraries were obtained. The results of assembly and clustering of ESTs were consisted of 2,693 unique sequences with 832 contigs (63%) and 1,861 singleton (37%). About 77% of genes could be assigned as known and putative functions using BLAST database (e-value: $<e^{-10}$). The expression patterns of two libraries were similar. Total 216 genes were up-regulated and 201 genes were down-regulated under low-temperature. The analysis of gene ontology (GO) generally showed similar pattern between two libraries with exception of a division of gene category. Most of cold regulated genes which were increased to 2 or 3 times than warm condition were belonging to stress response, polysaccharide bio-synthesis and antioxidant enzyme. On the other hand, phosphorylation and TCA cycle involved genes were down-regulated at low-temperature. The role of cold specific genes in *S. varians* are analyzed and discussed.

THE DIVERSITY OF HARPACTICOID COPEPODS IN THE POLAR REGIONS.

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ABSTRACT

Harpacticoid copepods are greatly diverse group, with over 4,300 species belonging to 589 genera and 56 families (Wells 2007). They are considered very important group because they are the second abundant group in the benthic habitats. In the polar, study on Harpacticoid copepods are relatively lack than other regions. So, we made a checklist of the polar Harpacticoid copepods. It is based on records in the literature and on our own data. There are about 203 species 99 genera 29 families of Harpacticoid copepods in the Arctic region (fig.1). Miraciidae is most specious family (36 species), followed by Ectinosomatidae (19) and Thalestridae (18). More than half of the species represented (109 Species) were encountered in the Svalbard archipelago. 109 species 66 genera 23 families of Harpacticoid copepods have been reported from the archipelago. In the region Franz Josep Land, 68 species 42 genera 16 families were reported. There are about 175 species 92 genera 26 species of Harpacticoid copepods in the Antarctic region (fig. 2). The most abundant species were reported in the Subantarctic region, 86 species 42 genera 21 families. We found 12 new species (undescribed) at 2002 and 2007 in Marian Cove, King George Island, Antarctic (fig. 3). Overall, the most numerous family is Miraciidae (17 species), followed by Canthocamptidae (16), Harpacticidae (15), Tisbidae (13), and Ectinosomatidae (12). There are about 375 species of Harpacticoid copepods in the polar region. The Arctic and the Antarctic have large unexplored habitats for Harpacticoid copepods. So we expect there are numerous new species of Harpacticoid copepods in the polar region.

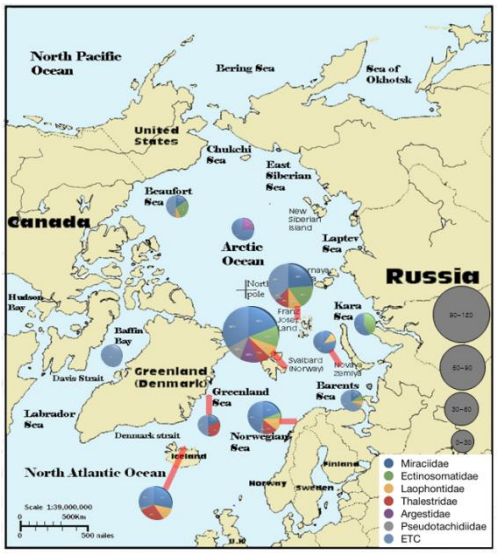

Fig. 1 *The distributions of Harpacticoid copepods in the Arctic.*

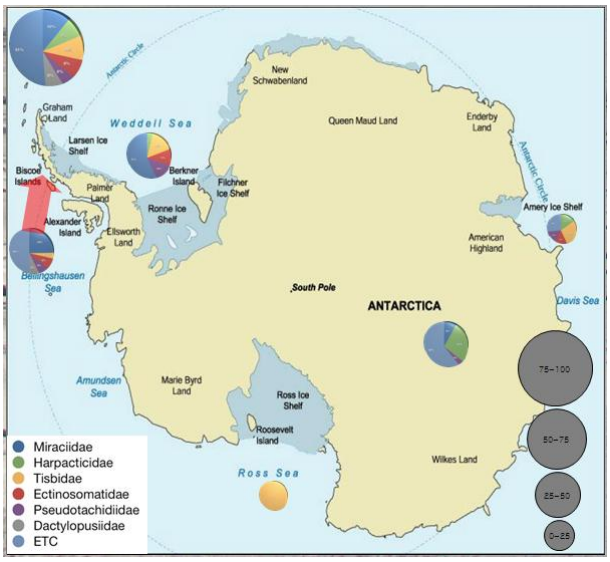

Fig. 2 *The distributions of Harpacticoid copepods in the Antarctic*.

Fig. 3 *New Harpacticoid copepods in Marian Cove, King George Island, Antarctic* (1. *Heteropsyllus sp., 2. Pseudobradya sp., 3. Argestidae gen. sp., 4. Sarsameira sp*.)

PERSISTENT TOXIC SUBSTANCES IN REMOTE LAKE AND COASTAL SEDIMENTS FROM SVALBARD, NORWAY: LEVELS, SOURCES AND FLUXES

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ABSTRACT

Within the framework of the Chinese Yellow River Station research monitoring program conducted in summer 2005, surface sediments from six lakes and two locations on the west coast of Spitsbergen near Ny-Å lesund, Svalbard, in the Norwegian Arctic were collected and concentrations of various groups of persistent toxic subtancces including polycyclic aromatic hydrocarbons (PAHs), polybrominated biphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were measured. Total PAH concentrations in lake sediments (mean: 260, range: 11 - 1100 ng/g dry wt) were higher than those previously reported in surface sediments collected from various lakes in Svalbard in 1995, suggesting that significant PAH contamination is occurring due to long-term atmospheric transport and the influence of local coal mining and fossil fuel sources around in the region of Ny-Å lesund. Interestingly, relatively high levels of PAHs were encountered from several lakes from Ny-Å lesund, which were within the range of levels reported for high-altitude mountain lakes i n Western and Central Europe and South America, as well as some urban/industr ialized areas in the world, pointing to the role of remote Arctic lakes as potentia l reservoirs of semi-volatile organic compounds, including PAHs. Compound-speci fic analysis revealed different PAH patterns between Ny-Å lesund lakes and Europ ean high mountain lakes, showing higher proportions of low molecular weight co mpounds and lower levels of high molecular weight PAHs in Norwegian Arctic l akes. PAH indicator ratios suggest that the majority of PAHs in lake sediments h ave pyrogenic origins (coal mining, fossil fuel and biomass combustion), while c oastal marine sediments were mainly contaminated by petroleum-derived PAHs (s hipping activities in coastal areas, and perhaps as a result of an oil spill in 198 6). Sediment fluxes of PAHs in Ny-Å lesund remote lakes were estimated to be 0. 2 - 22 ng cm^{-2} yr^{-1}, which were similar to those observed in Western and Centr al European high mountain lakes. These fluxes account for an accumulation rate of 286 mg yr^{-1} in the study lake sediments. The current PAH levels in sediments from three lakes exceeded Canadian sediment quality guidelines, suggesting the p resence of possible risks for aquatic organisms and the need for further studies.

THE EFFECTS OF LIGHT AND NUTRIENT ENRICHMENT ON THE PRIMARY PRODUCTIVITY IN THE CANADA BASIN

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ABSTRACT

The Joint Ocean Ice Study (JOIS) was conducted in the Canada Basin from mid September to mid October in 2009. During the period, the primary productivity of phytoplankton was measured at six different light depths of 11 stations in the Canada Basin, using a ^{13}C-^{15}N dual isotope tracer. In addition, we identified the effects of light and nutrient enrichments on the primary production of phytoplankton in the chlorophyll a maximum layer. The temperature and salinity at surface were -2~ 1℃ and 24-27, respectively in the Canada Basin. In contrast, the very salty water (>31) was existed below 60m water depth and the strong stratification was developed at the depth. In general, the NO_3^- concentration was depleted from surface to 60m over the North of 72^0. The primary productivity of phytoplankton was somewhat lower in the Canada Basin during the cruise period in 2009 compared to other regions in the Arctic Ocean. The averaged hourly primary production rate vertically integrated from 100% to 1% light depth was 1 mg C m^{-2} h^{-1} from this cruise. The light and nutrient enrichments induced higher primary productivity of the phytoplankton in the chlorophyll a maximum layer. The increases of primary productivity at the stations with <50% ice cover were much higher than those from other stations with >50% ice cover. This is probably because the phytoplankton at the stations with the higher ice cover was more shade-adapted and thus slower response on the light enrichments.

INTRODUCTION

Most of the Canada Basin is surrounded with first-year ice and/or multi-year ice. And the Canada Basin is one of the least known areas in the Arctic Ocean (Lee and Whitledge, 2005). The Canada Basin water column contains three main layers: a low-salinity upper layer, which includes a mixed layer and halocline; a warm Atlantic layer; and a cold, more saline deep layer (McLaughlin et al., 2005). Pacific-origin waters comprise most of the halocline and overlie the more saline Atlantic-origin component (McLaughlin et al., 2005). Macdonald et al. (1999) suggested that increased ice-melt, a thickening of the Atlantic layer, a decrease in thickening of the Pacific-origin halocline and an increased volume of Atlantic-origin waters that had entered via the Barents Sea were observed in the southern Canada Basin. Recently, these physical environmental changes of the Canada Basin will affect on the production of primary producer such as

phytoplankton, ice algae.

MATERIALS AND METHODS

In-situ carbon and nitrogen uptake rates of phytoplankton were measured at 11 stations in the Canada Basin, using both ^{13}C-^{15}N dual tracer techniques. To identify effects of light and nutrient on the carbon uptakes of phytoplankton, we took a water sample of chlorophyll a maximum layer. For the light enrichment experiments, we sampled in polycarbonate incubation bottles (0.5L) with mesh screen which light penetration controlled and we added labeled carbon ($NaH^{13}CO_3$). In the nutrient enrichment experiments, we sampled in polycarbonate incubation bottles (1L) and then added nitrate standard solution (KNO_3: 1, 5, 10 mol) and labeled carbon ($NaH^{13}CO_3$). Samples are incubated in the water bath for 3 or 4 hours. Particulate organic carbon and nitrogen and abundance of ^{13}C and ^{15}N were determined in the Finnigan Delta+XL mass spectrometer at University of Alaska Fairbanks after HCl fuming overnight to remove carbonate.

RESULTS AND DISCUSSION

The averaged hourly primary production rate vertically integrated from 100% to 1% light depth was 1 mg C m^{-2} h^{-1} from this cruise (Fig. 1). The primary productivity of phytoplankton was somewhat lower in the Canada Basin during the cruise period in 2009 compared to other regions in the Arctic Ocean.

The light and nutrient enrichments induced higher primary productivity of the phytoplankton in the chlorophyll a maximum layer. The increases of primary productivity at the stations with <50% ice cover were much higher than those from other stations with >50% ice cover (Fig. 2). This is probably because the phytoplankton at the stations with the higher ice cover was more shade-adapted and thus slower response on the light enrichments.

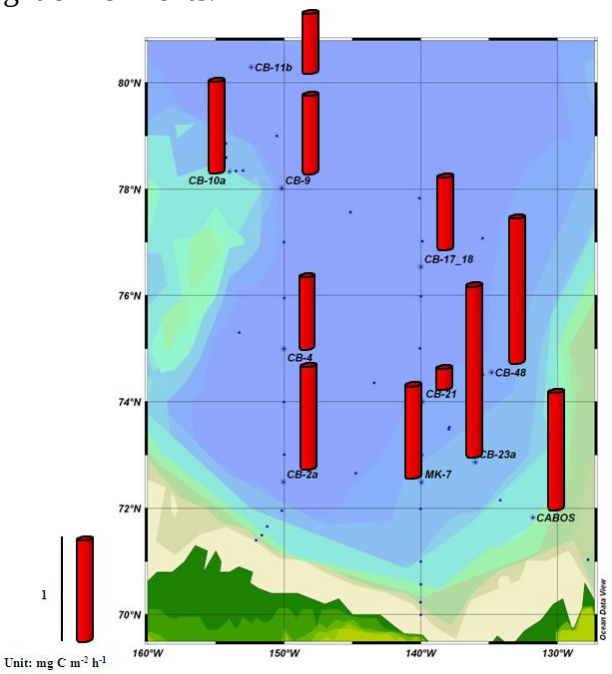

Fig. 1. *Carbon uptakes at the productivity stations in the Canadian basin in 2009.*

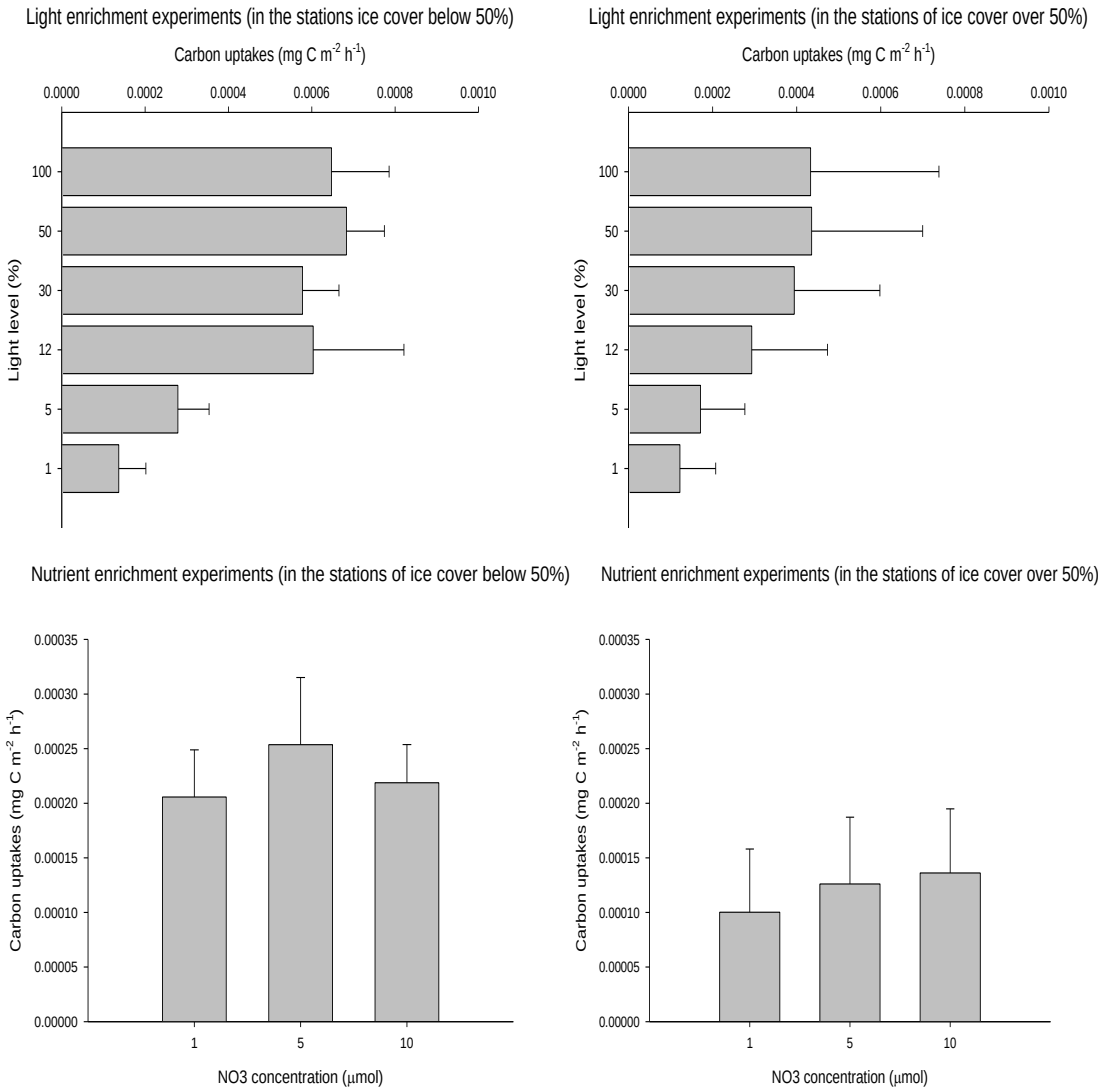

Fig. 2. *Specific carbon uptakes depending on different sea ice concentrations in the light and nutrient enrichment experiments.*

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PROTEIN ADAPTATION IN POLAR ENVIRONMENT: A COMPARATIVE STUDY OF A-TUBULIN SEQENCES IN MESOPHILIC AND PSYCHROPHILIC POLAR MICROALGAE

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Although microtubules usually disassemble at low temperature below 4℃ by disturbing polymerization of tubulin dimer, organisms living in a cold environment seem to overcome this limit by amino acid substitution of tubulin. We aimed to investigate whether amino acid substitution on tubulin occur commonly in polar microalgae and is responsible for promoting growth at freezing temperature at which non-cold adapted algae seldom grow and eventually die. The full-length cDNAs of α-tubulin from eight genera with 14 microalgal strains (ArF04, ArF08, ArF13, ArF24, ArF25, ArF26, ArF27, ArF28, ArF29, ArF32, AnF48, AnM08, AnM30, and AnM45) collected from the Arctic and the Antarctic were obtained by RACE and compared them with that of α-tubulin from mesophilic alga, *Chlamydomonas reinhardtii*. We found that the several amino acid substitutions occurred in most tubulins of polar microalgae. These substitutions were found in overlapped sites reported in previous work, presumably due to selective pressure of cold environment. Of the substituted sequences, the A295V region was conspicuous, which has reported that play an important role in the producing protofilament due to the increasing hydrophobicity. 10 out of 14 microalgal strains (72%) showed a substitution of alanine (A) to valine (V) in the 295th residue demonstrating that the substitution of A295V region is a largely conserved feature among polar microalgae. The substitutions within tubulin sequences may increase the polymerization of tubulin dimer, consequently turning on a signal transduction pathway involved in microtubule-mediated cell polarization etc, which increase the survival in freezing environment such as polar region. Our data will provide the valuable information to dissect the cold-adaptive mechanism related to structural change and/or post-translational modification of tubulin.

MOLECULAR CHARACTERIZATION OF COLD RESPONSIVE PROTEIN ANF48_RPL11 FROM ANTARCTIC MICRO GREEN ALGA ANF48

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A polar micro green alga, AnF48 was collected from near the King Sejong Station located in King George Island in the Antarctica (62° 13′S 58° 47′W). It is likely an endemic species, closely related to the genus *Hematococcus* based on 18S and *rbc*L sequence data. AnF48 successfully proliferated and propagated near 4-10℃, in contrast it showed dramatic growth decrease (up to 50%) when the ambient temperature exceeded 15℃. To discover the genes which are responsible for this specific temperature adaptation, GeneFishing DEG PCR was performed using cDNAs extracted from AnF48 grown at different temperatures (4℃, 15℃, 25℃). Genes induced by low temperature were identified by cloning and sequencing. One of them was a homolog of RPL11 which has been reported as a cold-responsive gene in *E. coli*. Thus, we named it AnF48_RPL11 (ribosomal protein L11). RT-PCR analysis confirmed that the expression level of AnF48_RPL11 was significantly up-regulated under 4℃ compared with that at 15℃ or 25℃. The full length cDNA sequences of AnF48_RPL11 was obtained using 5' and 3' race. The open reading frame consisted of 544 bp encoding 125 amino acids. It contains four exons and three introns meanwhile *Chlamydomonas reinhardtti* has seven exons and six introns. To our surprise, the similarity of deduced amino acids of AnF48_RPL11 is higher with RPL11 in *Arabidopsis thaliana* (92%) than in algal homologues *Chlamydomonas reinhardtii* (88%). This results suggested cold adaptative mechanism is conserved in a wide range of organism from *E. coli* to higher plant. We first report RPL11 from Antarctic micro green alga and provide molecular evidence for evolutionary conservation upon cold stimuli.

APOPTOTIC AND ANTI-INFLAMMATORY EFFECT OF METHANOLIC EXTRACT FROM FRESHWATER GREEN ALGA, *SPIROGYRA VARIANS* IN CHONDROCYTES AND CANCER CELL

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Apoptosis, anti-inflammatory and dedifferentiation effects of methanolic extracts from a total of 13 species (10 of green, two of red and one of brown algae) of algae which were collected from arctic region and Korea in winter were screened. Among screened algae, only two species, *Spirogyra varians* and *Spirogyra distenta*, showed strong effect for dedifferentiation, apoptosis and anti-inflammatory property in chondrocytes (primary cultured) and human cancer cell (A549 and MDAMB231-p53 null). Death rate of screened cell line dropped to 50% by treatment of methanolic extracts (5 μg/mL). The expression of type II collagen that senses induction of dedifferentiation was strongly inhibited, on the other hand, Extracts (5 μg/mL) from *Spirogyra* promoted up-regulation of COX-2 and p53 protein in rabbit chondrocytes. A549 and MDAMB231 cell also showed similar effect with chondrocytes cell. Treatment of extract induced increment of COX-2 against A549 cell while treatment against MDAMB231 cell induced increment of COX-2 and p53.

PLASTID TRANSFORMATION OF AN ARCTIC MOSS, AULACOMNIUM TURGIDUM

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The plastid transformation approach offers a number of unique advantages, including high-level transgene expression, multi-gene engineering, and a lack of gene silencing and position effects. The extension of plastid transformation technology to nonvascular plants, including mosses and liverworts, bears great promise for the understanding primitive system of environmental adaptation, and the efficient production of pharmaceutical metabolites. Here, we report a promising step towards stable plastid transformation in an arctic moss *Aulacomnium turgidum*. We produced transplastomic *A. turgidum* colonies and demonstrated transmission of the plastid expressed spectinomycin resistance gene (*aadA*). Transgenic chloroplasts were determined by PCR and genomic Southern analyses. Although the produced rice plastid transformants were found to be heteroplastomic, and the transformation efficiency requires further improvement, this study has established a variety of parameters for the use of plastid transformation technology in mosses and polar organisms.

SINGLE NUCLEOTIDE POLYMORPHISMS BASED PHYLUM SPECIFIC PCR AMPLIFICATION TECHNIQUE (SPAT): ITS APPLICATION TO ANALYZE POLAR MICROBIAL EUKARYOTIC COMMUNITY

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Over the past several decades, many genes such as SSU rDNA were successfully used to resolve ambiguity of traditional morphology-based taxonomy. The next challenge in population genetics and molecular evolution is to investigate the diversity of eukaryotes at the community level using high-throughput sequencing technique. However, even though high-throughput sequencing technique has a powerful potential to study community diversity, it has not been successful to apply the technique to study eukaryotic community due to biased PCR enrichment of dominant species in the community. In this study, we found several taxon specific SNP sites from monophyletic phyla such as ciliates and diatoms which are most dominant in marine ecosystem. By using these taxon specific SNP sites, we developed SNP-based phylum specific PCR Amplification Technique (SPAT). We observed that our method has a higher specificity compared to traditional universal primer based PCR amplification method. We successfully characterize the diversity of diatoms and ciliates without isolation process of them from polar eukaryotic community. Combining phylum specific PCR amplification method with pyrosequencing technique, it will be feasible to monitor the change of the community structure of these important marine protists at the seasonal and annual level.

PHYTOPLANKTON PRODUCTIVITY IN THE RUSSIAN CHUKCHI SEA IN 2009

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ABSTRACT

It is very important to study the Chukchi Sea and Arctic Ocean in order to understand the global marine ecosystems responding to the current climate changes, but there have been not much study because of the difficulty in logistics. The RUSALCA cruise in 2009 provided very important opportunities to research marine environments and ecosystems in the Russian and US sides of the Chukchi Sea. The main objectives were to measure primary productivity of phytoplankton and understand which controlling factors are important for the phytoplankton growth in the Chukchi Sea. The light intensity at the highest peak in a day ranged from 200 μE m^{-2} s^{-1} to 1200 μE m^{-2} s^{-1} at around 4 pm depending on weather at that time when they were measured. Prochlorococcus and Synechococcus found first in the Chukchi Sea contributed about 30% in the cell abundance of small phytoplankton community (< 20 μm). Integrated chlorophyll-a concentrations were relatively low (< 100 mg chl-a m^{-2}) in the Chukchi Sea this year. The average of integrated chlorophyll-a concentrations was 57.7 mg chl-a m^{-2} (± 37.8 mg chl-a m^{-2}), which was 3 times lower than that (155.6 mg chl-a m^{-2}) in 2004 (Lee et al. 2007). In consistent, the average of the carbon production rate was 17.03 mg C m^{-3} h^{-1}, which was also 2 fold lower than that in 2004 (Lee et al. 2007).

INTRODUCTION

The Bering Sea supports high rates of primary production and extraordinary rich marine resources, which are valuable fisheries, and immense populations of marine birds and mammals. Recently, major changes in Bering Sea stocks of phytoplankton, zooplankton, and commercially important ground fish, as well as marine mammals and seabirds have correlated with temporal shifts in environmental characteristics and physical forcing mechanisms (Livingston et al. 1999). Schell (2000) hypothesized that there has been a 30-40 % decrease in seasonal primary productivity in the northern Bering Sea over several decades. However, there has been no research to support his hypothesis for the decline in the productivity in this region since the ISHTAR and BERPAC programs in the late 1980s. In the Chukchi Sea, recently, Lee et al. (2007) found that the integrated concentration of the high nitrate pool in the central Chukchi Sea was greater in their study than in previous studies although the highest nitrate concentration (~ 22 μM) in the Anadyr Water mass passing through the western side of Bering Strait was consistent with prior observations. In contrast chlorophyll-a concentrations were lower than those from previous studies. They also found that the carbon and nitrogen production rates of phytoplankton were consistently 2 or 3 fold

lower than those from previous studies. However, they do not know if the lower rate of production from this study is a general decreasing trend or simply temporal variations in the Chukchi Sea, since temporal and geographical variations are substantially large and presently unpredictable. It is therefore particularly important to keep monitoring the Chukchi Sea insofar as processes there both reflect changes in the Bering Sea and regional biological production rates in the Pacific Arctic Ocean (Walsh et al. 1989; Walsh et al. 1997).

RESULTS AND DISCUSSION

Prochlorococcus and Synechococcus found first in the Chukchi Sea contributed about 30% in the cell abundance of small phytoplankton community (< 20 μm). In general, Synechococcus was more abundant in the southern part whereas Prochlorococcus was more abundant in the northern part of the study area in the Chukchi Sea in 2009. In contrast, eukaryotes such as pico and nano phytoplankton contributed about 70 % of the community (Fig .1). Integrated chlorophyll-a concentrations were relatively low (< 100 mg chl-a m^{-2}) in the Chukchi Sea in 2009 (Fig. 2). Generally, the highest concentrations were in the central southern part of the Chukchi Sea, but these values in 2009 were much lower than those measured in previous studies. The average of integrated chlorophyll-a concentrations was 57.7 mg chl-a m^{-2} (± 37.8 mg chl-a m^{-2}), which was 3 times lower than that (155.6 mg chl-a m^{-2}) in 2004 (Lee et al. 2007). In consistent, the production rates of phytoplankton generally low in the Chukchi Sea in 2009 (Fig. 2). The production rate was highest at the station CL6 (59.26 mg C m^{-3} h^{-1}) in the central southern part of the Chukchi Sea, whereas the lowest rate was at GD7 (0.57 mg C m^{-3} $h^{-1)}$ in the northern part of the Chukchi Sea in 2009. The average of the carbon production rate was 17.03 mg C m^{-3} h^{-1}, which was also 2 fold lower than that in 2004 (Lee et al. 2007).This lower rate might be a seasonal or inter-annual variation of phytoplankton in this region.

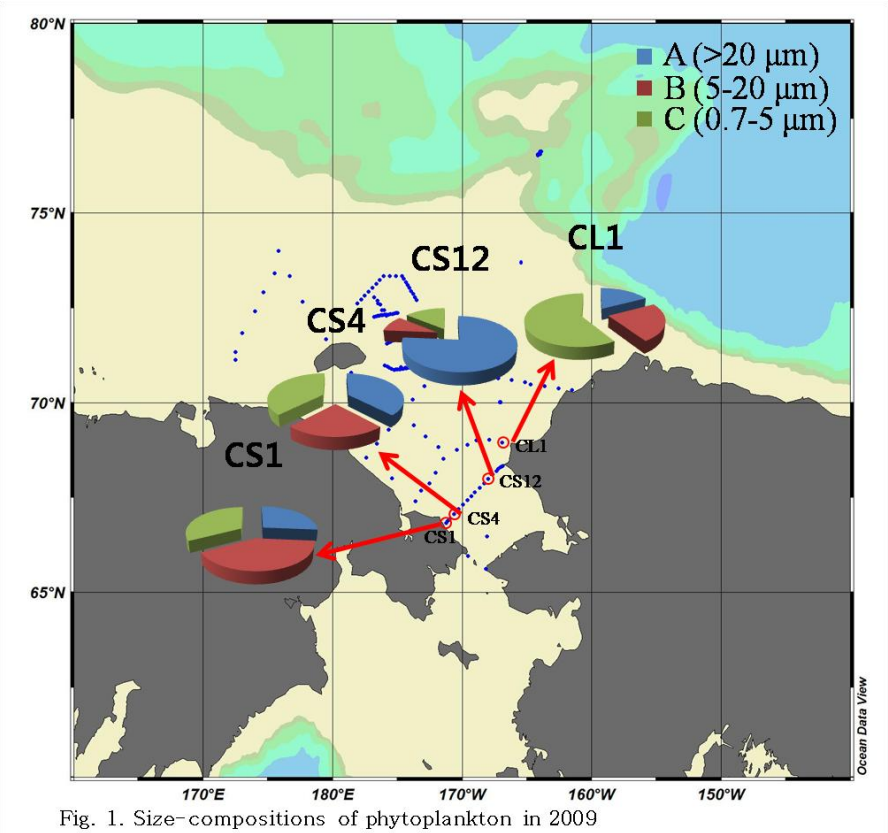

Fig. 1. Size-compositions of phytoplankton in 2009

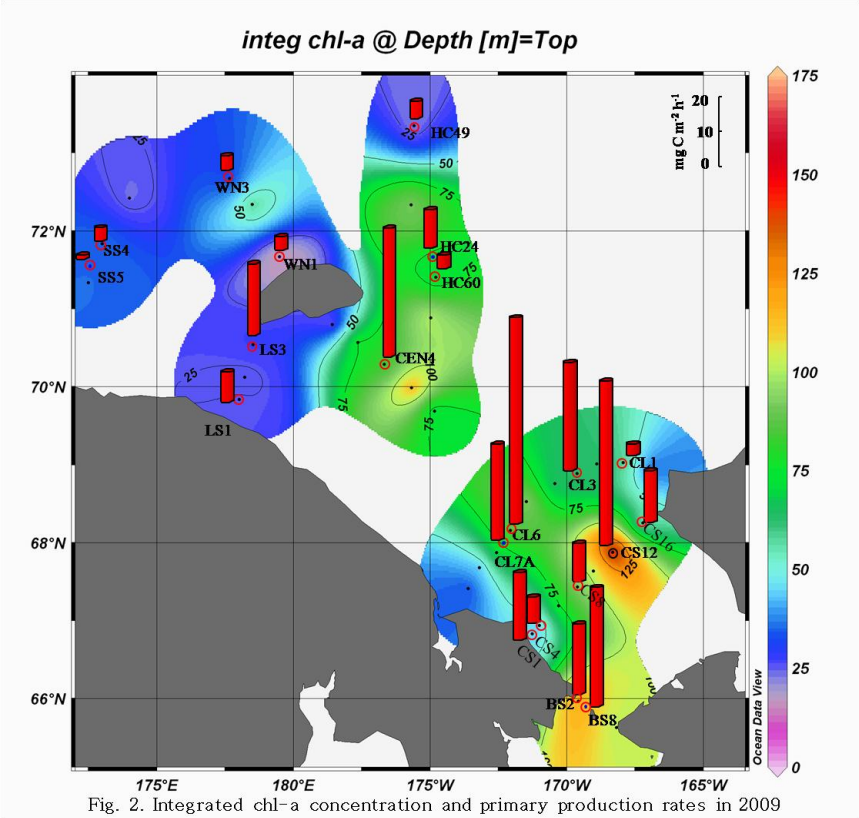

Fig. 2. Integrated chl-a concentration and primary production rates in 2009

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UV- ABSORBING COMPOUNDS (MYCOSPORINE LIKE AMINO ACIDS) DISTRIBUTION AND UPTAKE RATE IN DASAN STATION, ARCTIC REGIONS

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ABSTRACT

We investigated the change in UV-absorbing compounds(Mycosporine like amino acids) using 13C tracer for effect of UV radiation to *in situ* phytoplankton at Ny-Alesund of Spitsbergen Island in DaSan station, Arctic Ocean during May, 2009. And we measured Mycosporine like amino acids (MAAs) distribution around Ny-Alesund of Spitsbergen Island Sea. 5 kinds of MAAs such as mycosporine-glycine, shinorine, porphyra-334, palythine and asterine-330 were detected in Arctic around seawater. *In situ* phytoplankton incubation was carried out 3 times for 72hours under UV radiation transmission in quartz bottles and a little transmission in polycarbonate bottles. Carbon uptake rate of *in situ* incubation did not show significant different between quartz bottles and PC bottles. Total MAAs production rate showed similar values of exposed UV and PAR. When we compared MAAs distribution around Arctic seawater, Shinorine and Palythine analogous distribution was found at in all station, but mycosporine-glycine showed unique distribution compared to other MAAs compounds. Mycosporine-glycine exhibited a similar distribution related to *phyaeocystis* sp. blooming area in Arctic seawater.

REDESCRIPTION OF *LAACKMANNIELLA NAVICULAEFERA* (LAACKMANN, 1907) KOFOID&CAMPBELL, 1929 (CILIOPHORA: TINTINNIDA) FROM ANTARCTIC WATER.

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Laackmanniell naviculaefera was collected from Antarctic water and stained with protargol impregnation. Until now, only 17 tintinnids species were recorded with protargol staining method. *L. naviculaefera* has been studied only its lorica structure. This study is first record of the infraciliature structure from genus *Laackmanniella*. The morphological data show 17-18 of collar membranelles and 29-32 of somatic kineties.

IN VITRO ANTIOXIDANT ACTIVITY OF RAMALIN FROM ANTARCTIC LICHEN *RAMALINA TEREBRATA* IN MURINE MACROPHAGE RAW 264.7 CELLS

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Excessive reactive oxygen species (ROS) are associated with cell damage and the body has developed defense mechanisms to minimize free radical-induced cell damage. Antioxidants play a key role in these mechanisms. Ramalin, a novel antioxidant compound, has been found in the Antarctic lichen *Ramalina terebrata.* In the present study, Ramalin was assessed to determine its antioxidant activity *in vitro*. Ramalin (1 μg/ml) significantly reduced the released nitric oxide (NO) and hydrogen peroxide in LPS (lipopolysaccharide)-stimulated murine macrophage RAW 264.7 cells. Macrophage cells are a part of critical defense mechanisms of immune system, but cause a number of problems when the cells are abnormally stimulated by excessive oxidative stress. Therefore, antioxidants such as Ramalin may serve as a ROS regulator. In conclusion, the present study shows that Ramalin from Antarctic *lichen Ramalin terebrata* has significant antioxidant activity indicating its high scavenging activity against nitric oxide and superoxide free radicals. Consequently, we suggest that Ramalin can be a strong therapeutic candidate for controlling oxidative stress in cells.

Keywords: Ramalin, ROS, Antarctic, macrophage, antioxidant

CARBON DYNAMICS OF FOREST FLOOR AND STEM IN BLACK SPRUCE FOREST SOILS, INTERIOR ALASKA

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Our automated open/close chamber system (AOCC) consists of eight chambers, a pump, CO_2 gas analyzer, and a datalogger for CO_2 data on the lichen, tussock, feather moss, and sphagnum moss of a black spruce forest, Interior Alaska, during the growing seasons of 2007 and 2008. During the observing periods of 2007 and 2008, the seasonal NEE was 0.127±0.049 and -0.039±0.025 $mgCO_2/m^2/s$ in tussock regime, and 0.006±0.011 and 0.028±0.017 $mgCO_2/m^2/s$ in sphagnum moss, respectively. Air temperature is a more significant regulator than soil temperature in determining the GPP and Re of forest floor vegetations. Air temperature explained 77–95% of the variability in GPP and Re of the floor vegetations. The contributions (%) of simulated seasonal GPP to the black spruce forest during non-growing periods (DOY 1–120 and 244–365) and during the growing period (DOY 121–243) of 2007 are 63–72%, 20–25%, and 8–18%, respectively. This indicates that the floor CO_2 exchange, as well as the contribution of winter carbon emission, is a component of the regional carbon budget that cannot be neglected. As the result of simulated GPP and Re in tussock during 2007, tussocks are found to have on atmospheric CO_2 release, similar to results of observation for 63-day of 2007.

On the other hand of stem respiration rates of black spruce (*Picea Mariana*), the continuous measurement of stem respiration was conducted in black spruce stands of different ages (4.3 to 13.5 cm in DBH) in Interior Alaska during the growing seasons of 2007 and 2008, using a pump, CO_2 analyzer, chambers, and data-logger. The averaged whole stem respiration rate is 0.011±0.005 $mgCO_2/m^2/s$ (range 0.005±0.002 to 0.015±0.008 $mgCO_2/m^2/s$, CV 45%) in black spruce stands, indicating remarkably diurnal and seasonal variations of stem respiration among the stems during the growing season. It is found that metabolism exhibits 1.5-fold higher in the younger black spruce stand than in the older. Temperatures in the air and stem are significant regulators in determining stem respiration. The annual stem respiration rates simulated by Q_{10} value based on air temperature are 41.2 and 36.8 gC/m^2 during 2007 and 2008, respectively, which corresponds to 5.2 and 5.0% of ecosystem respiration and GPP during 2007. This suggests that stem respiration is a significant component in the scaling-up of the regional carbon budget in a black spruce forest, Interior Alaska. Quantification of the effects of regional change on the black spruce forest carbon balance and atmosphere-forest interactions requires a better understanding of respiration response.

CULTURED BACTERIAL DIVERSITY AND HUMAN IMPACT ON ALPINE GLACIER CRYOCONITE

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The anthropogenic effect on the microbial community in alpine glacier cryoconites was investigated by cultivation and physiological characterization of bacteria from six cryoconite samples with different amounts of human impact. Two hundred and fifty eight bacterial isolates were included in *Actinobacteria* (8%, particularly *Arthrobacter*), *Bacteroidetes* (14%, particularly *Olleya*), *Firmicutes* (2%), *Alphaproteobacteria* (2%), *Betaproteobacteria* (16%, particularly *Janthinobacterium*) and *Gammaproteobacteria* (58%, particularly *Pseudomonas*). Among them, isolates of *Arthrobacter* were detected only from samples with no human impact, while isolates affiliated with *Enterobacteriaceae* were detected only from samples with strong human impact. Gross physiological characteristics of each sample were affected by the frequency of human visits. Bacterial isolates included in *Actinobacteria* and *Bacteroidetes* were frequently isolated from pristine samples and showed low maximum growth temperature and enzyme secretion. Bacterial isolates included in *Gammaproteobacteria* were more frequently isolated from samples with stronger human impact and showed high maximum growth temperature and enzyme secretion. Ecotypic difference was not evident among isolates of *Janthinobacterium lividum*, *Pseudomonas fluorescens*, and *Pseudomonas veronii* which were frequently isolated from samples with a different degree of anthropogenic effect.

BACTERIAL COMPOSITIONS IN TWO ARCTIC DIATOM CULTURES DETERMINED BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) ANALYSIS

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Bacteria and phytoplankton are known to be tightly linked in marine ecosystem. However, information on bacterial compositions in phytoplankton cultures established from polar regions is still limited. To analyze bacterial diversity in two Arctic diatom cultures including *Fragilaria* sp. ArM 0014 and *Chaetoceros* sp. ArM 0035, denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was made. Six and 13 bacterial phylotypes were found in the cultures of *Fragilaria* sp. ArM 0014 and *Chaetoceros* sp. ArM 0035, respectively. For the culture of *Fragilaria* sp. ArM 0014, all bacterial phylotypes were affiliated with the class *Gammaproteobacteria*. For the culture of *Fragilaria* sp. ArM 0014, seven out of the 13 bacterial phylotypes were affiliated with the class *Gammaproteobacteria*. In addition, five and 1 bacterial phylotypes affiliated with the class *Bacteroidetes* and *Alphaproteobacteria*, respectively, were found in the culture of *Chaetoceros* sp. ArM 0035, which were not detected in the culture of *Fragilaria* sp. ArM 0014. Further studies will be required to find out which factors are important for harboring bacterial communities in polar phytoplankton.